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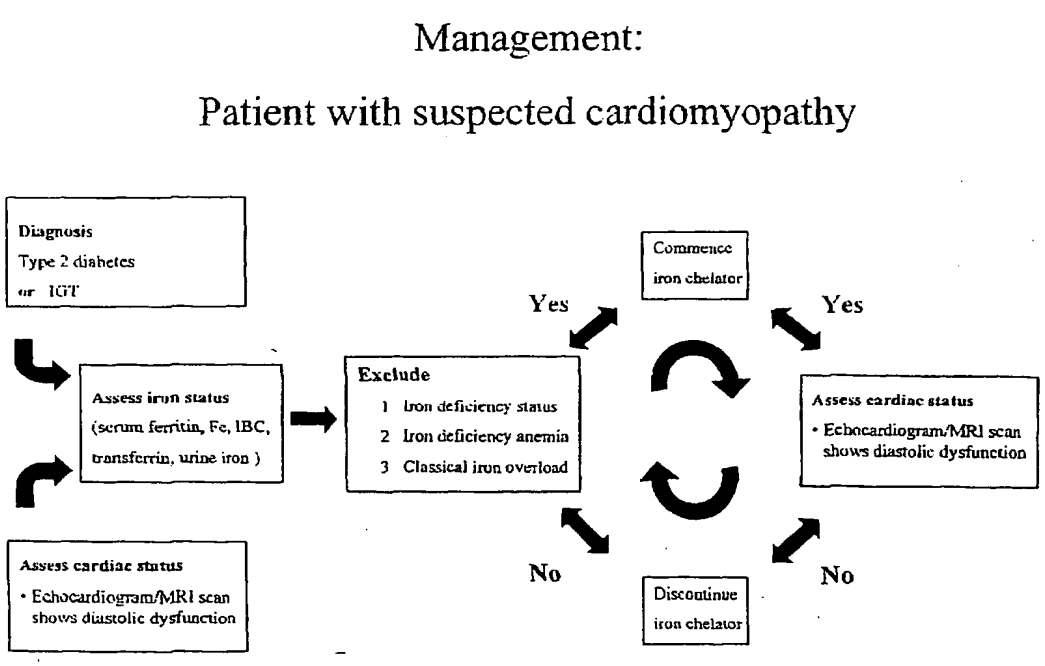

(57) Abstract: A method of improving tissue repair in a mammalian patient of damaged tissue selected from that of the myocardium, the vascular tree and organs dependent on the vascular tree, said method comprising or including the step of subjected the patient to, and/or administering to the patient, an agent or agents effective in lowering the iron values content of the patient's body sufficient to improve tissue repair.

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PREVENTING AND/OR TREATING VASCULAR DISEASE, CARDIOMYOPATHY AND/OR ASSOCIATED HEART FAILURE

FIELD OF THE INVENTION

This invention concerns methods of treatment, prevention or amelioration of a disease, disorder or condition (hereafter "treating") in a mammal, including, for example, a human being, predisposed to iron or iron and copper-involved or -mediated free radical tissue damage of tissue and/or to iron or iron and copper-involved or -mediated impairment of normal tissue stem cell responses. The invention has application *inter alia* to heart failure, macrovascular disease or damage, microvascular disease or damage, and/or toxic (*e.g.*, hypertensive) tissue and/or organ disease (such ailments as may, for example, be characterised by heart failure, cardiomyopathy, myocardial infarction, and related arterial and organ diseases) and to related compounds, formulations, uses and procedures.

BACKGROUND OF THE INVENTION

The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art, or relevant, to the presently described or claimed inventions, or that any publication or document that is specifically or implicitly referenced is prior art.

Glucose is the primary source of energy for the human body. Absorbed from the intestine it is metabolized by energy production (by conversion to water and carbon dioxide), conversion to amino acids and proteins or keto-acids, and storage as glycogen. Glucose metabolism is regulated by complex orchestration of hormone activities. While all dietary sugars are broken down into various carbohydrates, the most important is glucose, which is metabolized in nearly all cells of the body. Glucose enters the cell by facilitated diffusion (glucose transport proteins). This facilitated transport is stimulated very rapidly and effectively by an insulin signal (glucose transport into muscle and adipose cells is increased up to twenty fold). After glucose is transported into the cytoplasm, insulin then directs the disposition of it by conversion of glucose to glycogen, to pyruvate and lactate, and to fatty acids.

Diabetes mellitus is heterogeneous group of metabolic disorders, connected by raised plasma glucose concentration and disturbance of glucose metabolism with resulting hyper-glycemia. The hyperglycemia in diabetes mellitus generally results from defects in insulin secretion, insulin action, or both. Although its etiology has been clouded, the World Health Organization (WHO) has set forth a classification scheme for diabetes mellitus that includes type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, and other specific types of diabetes mellitus. Former terms like IDDM (insulin-dependent diabetes mellitus), NIDDM (non-insulin dependent diabetes mellitus), and juvenile-onset diabetes mellitus or adult-onset diabetes mellitus are no longer primarily used to describe those conditions.

The terms "insulin-dependent diabetes" (IDDM) or "juvenile-onset diabetes" previously encompassed what is now referred to as type 1 diabetes. Type 1 diabetes results from an autoimmune destruction of the insulin-secreting ß-cells of the pancreas. There are several markers of this autoimmune destruction, detectable in body fluids and tissues, including islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2ß. Genetic factors are strongly implicated. On the other hand, the concordance rate in twin studies is under 50% and supports a role for environmental factors including viral infections. The autoimmune process begins many years before clinical detection and presentation. The rate of ß-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and usually slow in adults.

The terms "non-insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes" previously encompassed what is now referred to as type 2 diabetes mellitus. The disease usually develops after 40 years of age. It is much more common that type 1 diabetes and comprises approximately 90% of all individuals with diabetes. Type 2 patients are usually older at the onset of disease, and exhibit various symptoms. Insulin concentrations are mostly increased but they can be normal or decreased. Obesity is common. Diet and exercise regimens leading to weight reduction can ameliorate hyperglycemia. Oral hypoglycaemic drugs are also used in an effort to

lower blood sugar. Nevertheless, insulin is sometimes required to correct hyperglycemia, particularly as patients grow older or as their ß-cells fail.

Two groups of disorders may be said to characterize type 2 diabetes mellitus. The first one is a decreased ability of insulin to act on peripheral tissues, usually referred to as "insulin resistance." Insulin resistance is defined as a decreased biological response to normal concentrations of circulating insulin and represents the primary underlying pathological process. The second is the dysfunction of pancreatic ß-cells, represented by the inability to produce sufficient amounts of insulin to overcome insulin resistance in the peripheral tissues. Eventually, insulin production can be insufficient to compensate the insulin resistance due to ß-cell dysfunction. The common result is a relative deficiency of insulin. Data support the concept that insulin resistance is the primary defect, preceding the derangement of insulin secretion. As with type 1 diabetes, the basis of the insulin resistance and insulin secretion defects is believed to be a combination of environmental and genetic factors.

Gestational diabetes mellitus is usually asymptomatic and generally not life threatening to the mother. The condition is associated with an increased incidence of neonatal morbidity, neonatal hypoglycaemia, macrosomia and jaundice. Even normal pregnancies are associated with increasing insulin resistance, mostly in the second and third trimesters. Euglycaemia is maintained by increasing insulin secretion. In those women who are not able to increase the secretion of insulin, gestational diabetes develops. The pathophysiology of gestational diabetes mellitus is not well known and includes family history of diabetes mellitus, obesity, complications in previous pregnancies and advanced maternal age.

Other specific types of diabetes mellitus are heterogeneous, with the following representing the largest groups: genetic defects of ß-cell function; genetic defects in insulin action; diseases of the exocrine pancreas (*e.g.*, pancreatitis, trauma/pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis, and others); other endocrinopathies (*e.g.*, acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma, and others); drug- or chemical-induced diabetes mellitus (*e.g.*, from vacor (an acute rodenticide

released in 1975 but withdrawn as a general-use pesticide in 1979 because of severe toxicity; exposure produces destruction of the beta cells of the pancreas, causing diabetes mellitus in survivors), pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, beta-adrenergic agonists, thiazides, phenytoin, alfa-interferon, and others); infection-induced diabetes mellitus (*e.g.*, from congenital rubella, cytomegalovirus, and others); rare forms of immune-mediated diabetes; and, other genetic syndromes sometimes associated with diabetes (*e.g.*, Down syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram syndrome, Friedreich's ataxia, Huntington's chorea, Lawrence-Moon Beidel syndrome, Myotonic dystrophy, Porphyria Prader-Willi syndrome, and others). The etiology and pathophysiology are very different, mostly complicated or connected to insulin secretion and action derangement, as well as signal transduction inside the cells disarrangement. See "The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Committee Report 2001," American Diabetes Association, *Diabetes Care* 1997;20:1183-97 (revised 1999; republished January 2002); Lernmark A., "Type I Diabetes," *Clin. Chem.* **45** (8B): 1331-8 (1999); Lebowitz H.E., "Type 2 Diabetes: An Overview," *Clin. Chem.* **45** (8B): 1339-45 (1999). The vast majority of cases of diabetes fall into two broad etiopathogenetic categories, type 1 diabetes (characterized by an absolute deficiency of insulin secretion) and the much more prevalent type 2 diabetes (characterized by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response).

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputation, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Glycation of tissue proteins and other macromolecules and excess production of polyol compounds from glucose are among the mechanisms thought to produce tissue damage from chronic hyperglycemia. Patients with diabetes have an

increased incidence of atherosclerotic cardiovascular, peripheral vascular, and cerebrovascular disease. Hypertension, abnormalities of lipoprotein metabolism, and periodontal disease are often found in people with diabetes.

Diabetes mellitus is a chronic condition characterized by the presence of fasting hyperglycemia and the development of widespread premature atherosclerosis. Patients with diabetes have increased morbidity and mortality due to cardiovascular diseases, especially for coronary artery disease. Vascular complications in diabetes may be classified as microvascular, affecting the retina, kidney and nerves and macrovascular, predominantly affecting coronary, cerebrovascular and peripheral arterial circulation. Chronic hyperglycemia results in hyperglycosylation of multiple proteins and is the hallmark of diabetes. Hyperglycosylated proteins have altered function resulting in a spectrum of effects.

Epidemiological studies have confirmed that hyperglycemia is the most important factor in the onset and progress of diabetes complications, both in insulin-dependent and non-insulin-dependent diabetes mellitus. Mechanisms connecting hyperglycemia with complications of long-term diabetes have been investigated and the involvement of non-enzymatic glycation processes is indicated. Nonenzymatic glycation is a process by which glucose is chemically bound to amino groups of proteins, but without the help of enzymes. It is a covalent reaction in which, by means of N-glycoside bonding, sugar-protein complex is formed through a series of chemical reactions described by Maillard. Maillard reactions are complex and multilayer, and can be analyzed in three stages. First, sugar-protein complexes are formed (Amadori rearrangement). It is an early product of nonenzymatic glycation, an intermediary which is a precursor of later compounds. The second stage includes the formation of numerous intermediary products among which some are very reactive and further continue with glycation reactions. The third, final phase, consists of complex product polymerization reactions which occurred in the second stage in the process of which heterogeneous structures called advanced glycation endproducts (AGE) are formed. It was believed that the primary role in Maillard reactions was exclusively played by high glucose concentration. However, recent data show that, in spite of the fact that

sugars are the main precursors of AGE compounds, numerous intermediary metabolites including a-oxoaldehydes also participate in nonenzymatic glycation reactions. Such intermediary products are generated during glycolysis (methylglyoxal) or in the polyolic pathway, and they can also be formed by autooxidation of carbohydrates (glyoxal). Alpha-oxoaldehydes modify AGEs surprisingly fast, in contrast to classical Maillard reactions which are slower.

Glycation has both physiological and pathophysiological significance. In physiological conditions glycation can be detected in the aging process, and the reactions are significantly faster and more intensive with frequently increased glucose concentrations. In diabetology the importance of these processes is manifest in two essential issues, the effect of protein glycation on the change of their structure and function, and the use of glycated proteins level as a parameter of integrated glycemia. A classical example of nonenzymatic glycation is the formation of glycated hemoglobin (more precisely, HbA1c). The degree of nonenzymatic glycation being directly associated with blood glucose levels, the percentage of HbA1c in diabetes can be very increased. HbA1c had been the first studied glycated protein, but it was soon discovered that other, various structural and regulatory proteins, are also subject to nonenzymatic glycation forming glycation end-products.

As noted above, during the glycation process early glycation products are formed first, which later rearrange into final AGE structures by a series of complex chemical reactions. Protein modification with AGE is irreversible, there being no enzymes in the organism able to hydrolyze AGE compounds, which consequently accumulate during the life span of a protein on which they had were formed. Examples include all types of collagen, albumin, basic myelin protein, eye lens proteins, lipoproteins and nucleic acid. AGEs change the function of many proteins and contribute to various late complications of diabetes mellitus. The major biological effect of excessive glycation include the inhibition of regulatory molecule binding, crosslinking of glycated proteins, trapping of soluble proteins by glycated extracellular matrix, decreased susceptibility to proteolysis, inactivation of enzymes, abnormalities of

nucleic acid function, and increased immunogenicity in relation to immune complexes formation.

It has also been well documented that AGEs progressively accumulate on the tissues and organs which develop chronic complications of diabetes mellitus like retinopathy, nephropathy, neuropathy and progressive atherosclerosis. Immunohistochemical methods have demonstrated the presence of different AGE compounds in glomeruli and tubuli cells in both experimental and human diabetic nephropathy. The AGE role in atherosclerosis may also be significant. For instance, reticulated and irreversible LDL from the circulation binds to AGE-modified collagen of blood vessel walls. In the majority of blood vessels such reticular binding delays normal outflow of LDL particles which penetrate vessel walls and thus enhance the deposit of cholesterol in the intima. This is followed by an accelerated development of atherosclerosis.

The level of AGE proteins reflects kinetic balance of two opposite processes, the rate of AGE compound formation and the rate of their degradation by means of receptors. AGE receptors participate in the elimination and change of aged, reticular and denaturated molecules of extracellular matrix as well as all other AGE molecules. However, in diabetes mellitus AGE protein accumulation may exceed the ability of their elimination due to chronic hyperglycemia and excessive glycation. AGE receptors were first detected on macrophage cells. AGE protein binding to macrophage cell receptors causes a cascade of events in the homeostasis of blood vessel walls and their milieu by mediation of cytokines and tissue growth factors. At least four different AGE receptors have been described, among which two belong to the group of receptor scavengers. One of them is very similar, if not identical, to the receptor that internalizes altered LDL particles. Receptors on endothelium cells differ. These are sites on cell membranes that bind AGE-ligands (denoted "RAGE" receptors). They belong to immunoglobulin receptor family and are prevalent in tissues. Binding of AGE compounds to RAGEs leads to cellular stress. It is not currently known whether variations in AGE level explain differences in susceptibility

to develop complications, but it has been theorized that gene diversity in AGE receptors could offer an explanation.

Hyperglycemia induces a large number of alterations in vascular tissue that potentially promote accelerated atherosclerosis. Currently, three major mechanisms have emerged that encompass most of the pathologic alterations observed in the vasculature of diabetic animals and humans: (a) nonenzymatic glycosylation of proteins and lipids; (b) oxidative stress; and (c) PKC activation. Importantly, these mechanisms are not independent. For example, hyperglycemia-induced oxidative stress promotes the formation of AGEs and PKC activation, and both type 1 and type 2 diabetes are independent risk factors for coronary artery disease (CAD), stroke, and peripheral arterial disease. Schwartz CJ, *et al.*, "Pathogenesis of the atherosclerotic lesion. Implications for diabetes mellitus," *Diabetes Care* 15:1156–1167 (1992); Stamler J, *et al.*, "Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial." *Diabetes Care* 16:434–444 (1993). Atherosclerosis accounts for virtually 80% of all deaths among North American diabetic patients, compared with one-third of all deaths in the general North American population, and more than 75% of all hospitalizations for diabetic complications are attributable to cardiovascular disease. American Diabetes Association, "Consensus statement: role of cardiovascular risk factors in prevention and treatment of macrovascular disease in diabetes," *Diabetes Care* 16:72–78 (1993). Sixteen million people in the United States are estimated to have diabetes, and more than 90% of these patients have type 2 diabetes. National Center for Health Statistics. *Health United Stats*. Washington, DC: Government Printing Office, 1998. The World Health Organization estimates that the number of diabetic adults will more than double globally, from 143 million in 1997 to 300 million in 2025, largely because of dietary and other lifestyle factors.

The decline in heart disease mortality in the general U.S. population has been attributed to the reduction in cardiovascular risk factors and improvement in treatment of heart disease. However, patients with diabetes have not experienced the reduction in age-adjusted heart disease mortality that has been observed in nondiabetics, and an

increase in age-adjusted heart disease mortality has been reported in diabetic women. Gu K, *et al.*, "Diabetes and decline in heart disease mortality in U.S. adults," *JAMA* 281:1291–1297 (1999). Studies have also shown that diabetic subjects have more extensive atherosclerosis of both coronary and cerebral vessels than age- and sex-matched nondiabetic controls. Robertson WB, Strong JP, "Atherosclerosis in persons with hypertension and diabetes mellitus," *Lab Invest* 18:538–551 (1968). It has also been reported that diabetics have a greater number of involved coronary vessels and more diffuse distribution of atherosclerotic lesions. Waller BF, *et al.*, "Status of the coronary arteries at necropsy in diabetes mellitus with onset after age 30 years. Analysis of 229 diabetic patients with and without clinical evidence of coronary heart disease and comparison to 183 control subjects," *Am J Med* 69:498–506 (1980). Large studies comparing diabetics with matched controls have also shown that diabetic patients with established CAD undergoing cardiac catheterization for acute myocardial infarction, angioplasty, or coronary bypass have significantly more severe proximal and distal CAD. Granger CB, *et al.*, "Outcome of patients with diabetes mellitus and acute myocardial infarction treated with thrombolytic agents. The Thrombolysis and Angioplasty in Myocardial Infarction (TAMI) Study Group," *J Am Coll Cardiol* 21:920–925 (1993); Stein B, *et al.*, "Influence of diabetes mellitus on early and late outcome after percutaneous transluminal coronary angioplasty," *Circulation* 91:979–989 (1995); Barzilay JI, *et al.*, "Coronary artery disease and coronary artery bypass grafting in diabetic patients aged > or = 65 years [report from the Coronary Artery Surgery Study (CASS) Registry]," *Am J Cardiol* 74:334–339 (1994)). Postmortem and angioscopic evidence also shows a significant increase in plaque ulceration and thrombosis in diabetic patients. Davies MJ, *et al.*, "Factors influencing the presence or absence of acute coronary artery thrombi in sudden ischaemic death," *Eur Heart J* 10:203–208 (1989); Silva JA, *et al.* "Unstable angina. A comparison of angioscopic findings between diabetic and nondiabetic patients," *Circulation* 92:1731–1736 (1995).

CAD is prevalent in both type 1 and type 2 diabetes. In type 1 diabetes, an excess of cardiovascular mortality can only be observed after the age of 30. Krolewski

AS, *et al.*, "Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus," *Am J Cardiol* 59:750–755 (1987). CAD risk was reported in this study to increase rapidly after age 40, and by age 55, 35% of men and women with type 1 diabetes die of CAD, a rate of CAD mortality that far exceeded that observed in an age-matched nondiabetic cohort. *Id.* Diabetic nephropathy in type 1 diabetics also increases the prevalence of CAD. Nephropathy leads to accelerated accumulation of advanced glycosylation end products (AGEs) in the circulation and tissue and parallels the severity of renal functional impairment. Makita Z, *et al.*, "Advanced glycosylation end products in patients with diabetic nephropathy," *N Engl J Med* 325:836–842 (1991). In diabetic patients reaching end-stage renal disease, overall mortality has been reported to be greater than in nondiabetic patients with end-stage renal disease. The relative risk for age-specific death rate from myocardial infarction among all diabetic patients during the first year of dialysis is 89-fold higher than that of the general population. Geerlings W, *et al.*, "Combined report on regular dialysis and transplantation in Europe, XXI," *Nephrol Dial Transplant* 6[Suppl 4]:5–29 (1991). The most common cause of death in diabetic patients who have undergone renal transplantation is also CAD, accounting for 40% of deaths in these patients. Lemmers MJ, Barry JM, "Major role for arterial disease in morbidity and mortality after kidney transplantation in diabetic recipients," *Diabetes Care* 1991;14:295–301 (1991).

With regard to people with type 2 diabetes, CAD is the leading cause of death, regardless of duration of diabetes. Stamler J, *et al.*, "Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial," *Diabetes Care* 1993;16:434–444; Donahue RP, Orchard TJ, "Diabetes mellitus and macrovascular complications. An epidemiological perspective," *Diabetes Care* 15:1141–1155 (1992). The increased cardiovascular risk is particularly striking in women. Barrett-Connor EL, *et al.*, "Why is diabetes mellitus a stronger risk factor for fatal ischemic heart disease in women than in men? The Rancho Bernardo Study," *JAMA* 265:627–631 (1991).

The degree and duration of hyperglycemia are the principal risk factors for microvascular complications in type 2 diabetes. The Diabetes Control and Complications Trial Research Group, "The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus," *N Engl J Med* 1993;329:977–986). However, there is no obvious association between the extent or severity of macrovascular complications and the duration or severity of the diabetes, and an increased prevalence of CAD is apparent in newly diagnosed type 2 diabetes subjects has been reported. Uusitupa M, Siitonen O, Aro A, *et al.*, "Prevalence of coronary heart disease, left ventricular failure and hypertension in middle-aged, newly diagnosed type 2 (non-insulin-dependent) diabetic subjects," *Diabetologia* 28:22–27 (1985). It has also been reported that even impaired glucose tolerance carries an increased cardiovascular risk despite minimal hyperglycemia. Fuller JH, *et al.*, "Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study," *Lancet* 1:1373–1376 (1980).

Insulin resistance is a common condition and, associated with genetic predisposition, sedentary lifestyle, and aging, it is exacerbated and produced by obesity. Thus, even in the absence of diabetes, insulin resistance is reportedly a major risk factor for CAD. Lempiainen P, *et al.*, "Insulin resistance syndrome predicts coronary heart disease events in elderly nondiabetic men," *Circulation* 100:123–128 (1999). Impaired insulin action coupled with compensatory hyperinsulinemia leads to a number of proatherogenic abnormalities referred to as insulin resistance syndrome. The association of insulin resistance with several established atherogenic risk factors reportedly promotes atherosclerosis many years before overt hyperglycemia ensues. Ferrannini E, *et al.*, "Insulin resistance in essential hypertension," *N Engl J Med* 1987;317:350–357; Zavaroni I, *et al.*, "Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance," *N Engl J Med* 320:702–706 (1989); Peiris AN, *et al.*, "Adiposity, fat distribution, and cardiovascular risk," *Ann Intern Med* 110:867–872 (1989); Reaven GM, "Role of insulin resistance in human disease (syndrome X): an expanded definition," *Annu Rev Med* 44:121–131 (1993).

The dyslipidemia associated with insulin resistance entails elevated very-low-density lipoprotein (VLDL)–triglyceride levels, low HDL levels, delayed postprandial clearance of triglyceride-rich lipoprotein remnants, and the presence of the very atherogenic, small, dense LDL particles. Grundy SM, "Hypertriglyceridemia, atherogenic dyslipidemia, and the metabolic syndrome," *Am J Cardiol* 81:18B–25B (1998). This atherogenic lipoprotein phenotype is the most common lipoprotein abnormality seen in patients with CAD and imparts a risk for CAD at least equal to that of isolated moderate to severe hypercholesterolemia. Austin MA, *et al.*, "Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk," *Circulation* 82:495–506 (1990). Insulin-resistant subjects also exhibit endothelial dysfunction and a hypercoagulable state, and chronic subclinical inflammation has emerged as part of the insulin-resistance syndrome. C-reactive protein, a marker of inflammation associated with cardiovascular events, is independently related to insulin sensitivity. Festa A, *et al.*, "Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS)," *Circulation* 102:42–47 (2000). The proatherogenic metabolic risk factors in insulin-resistance subjects worsen continuously across the spectrum of glucose tolerance. Meigs JB, *et al.*, "Metabolic risk factors worsen continuously across the spectrum of nondiabetic glucose tolerance. The Framingham Offspring Study," *Ann Intern Med* 128:524–533 (1998). Whether compensatory hyperinsulinemia promotes atherosclerosis in insulin-resistant subjects is not clear.

The atherogenic risk factor profile observed in insulin-resistance patients accounts for only a portion of the excess risk for CAD in patients with type 2 diabetes, indicating that hyperglycemia itself plays a central role in accelerating atherosclerosis in these patients. Thus, insulin-resistant individuals who go on to develop type 2 diabetes become exposed also to the atherogenic effects of hyperglycemia. Furthermore, the threshold above which hyperglycemia becomes atherogenic is unknown but may be in the range defined as impaired glucose tolerance. Gerstein HC, Yusuf S, "Dysglycaemia and risk of cardiovascular disease," *Lancet* 347:949–950 (1996). Various population-based studies in patients with type 2 diabetes are reported

to have shown a positive association between the degree of glycemic control and CAD morbidity and mortality in middle-aged and elderly type 2 diabetic subjects. Turner RC, *et al.*, "Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23)," *BMJ* 316:823–828 (1998); Kuusisto J, *et al.*, "NIDDM and its metabolic control predict coronary heart disease in elderly subjects," *Diabetes* 43:960–967 (1994); Laakso M, "Hyperglycemia and cardiovascular disease in type 2 diabetes," *Diabetes* 48:937–942 (1999).

The metabolic abnormalities associated with types 1 and 2 diabetes also result in profound changes in the transport, composition, and metabolism of lipoproteins. Lipoprotein metabolism is influenced by several factors including type of diabetes, glycemic control, obesity, insulin resistance, the presence of diabetic nephropathy, and genetic background. Ginsberg HN, "Lipoprotein physiology in nondiabetic and diabetic states. Relationship to atherogenesis," *Diabetes Care* 14:839–855 (1991). Abnormalities in plasma lipoprotein concentrations are commonly observed in diabetic individuals and contribute to the atherosclerotic process. The level of glycemic control is the major determinant of lipoprotein levels in type 1 diabetic patients. Garg A, "Management of dyslipidemia in IDDM patients," *Diabetes Care* 17:224–234 (1994). In well- to moderately-controlled diabetes, lipoprotein levels are usually within the normal range, while in poorly controlled type 1 diabetic patients, triglycerides are markedly elevated, LDL is modesty increased (usually when HbA_{1c} is greater than 11%), and HDL levels are decreased. In contrast to type 1 diabetes, the pathophysiology of dyslipidemia in type 2 diabetes results from a complex relationship between hyperglycemia and the insulin-resistance state. The typical lipoprotein profile associated with type 2 diabetes includes high triglycerides, low HDL levels, and normal LDL levels, the most consistent change being an increase in VLDL-triglyceride levels. Syvanne M, Taskinen MR, "Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus," *Lancet* 350:SI20–SI23 (1997); Ginsberg HN, "Diabetic dyslipidemia: basic mechanisms underlying the common hypertriglyceridemia and low HDL cholesterol levels," *Diabetes* 45[Suppl

3]:S27–S30 (1996). HDL levels are typically approximately 25% to 30% lower than in nondiabetic subjects and are commonly associated with other lipid and lipoprotein abnormalities, particularly high triglyceride levels.

Hypertriglyceridemia in type 2 diabetes results from high fasting and postprandial triglyceride-rich lipoproteins, especially Very-Low-Density Lipoprotein (VLDL). Type 2 diabetic subjects with hypertriglyceridemia have both overproduction and impaired catabolism of VLDL. Increased VLDL production is almost uniformly present in patients with type 2 diabetes and hypertriglyceridemia. Increased VLDL production in diabetes is a consequence of an increase in free fatty acid mobilization (because maintenance of stored fat in adipose tissue depends on the suppression of hormone-sensitive lipase by insulin) and high glucose levels. Because free fatty acid availability is a major determinant of VLDL production by the liver, VLDL overproduction and hypertriglyceridemia occur.

The rest of the dyslipidemic phenotype that characterizes insulin resistance and type 2 diabetes (low HDL and small, dense LDL) – which has been termed atherogenic lipoprotein phenotype (Austin MA, *et al*, "Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk," *Circulation* 82:495–506 (1990)) – follows once VLDL secretion increases, mainly through the action of cholesteryl ester transfer protein and lipoprotein compositional changes that occur in plasma. Ginsberg HN, "Insulin resistance and cardiovascular disease," *J Clin Invest* 106:453–458 (2000). Increased fatty acid flux to the liver also results in the production of large triglyceride-rich VLDL particles because the size of VLDL is also mainly determined by the amount of triglyceride available. VLDL size is an important determinant of its metabolic fate. Large triglyceride-rich VLDL particles may be less efficiently converted to LDL, thereby increasing direct removal from the circulation by non-LDL pathways. In addition, overproduction of large triglyceride-rich VLDL is associated with the atherogenic small, dense LDL subclass.

In type 2 diabetic subjects with more severe hypertriglyceridemia, VLDL clearance by lipoprotein lipase (LPL) – the rate-limiting enzyme responsible for the removal of plasma triglyceride-rich lipoproteins – is also impaired. Syvanne M,

Taskinen MR, "Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus," *Lancet* 350:SI20–SI23 (1997). LPL requires insulin for maintenance of normal tissue levels, and its activity is low in patients with poorly controlled type 2 diabetes. The result is enzymatic activity insufficient to match the overproduction rate, with further accumulation of VLDL triglyceride. Triglyceride concentrations are associated with premature CAD, and studies have shown that triglyceride-rich lipoproteins play an important role in the progression of atherosclerosis. Hodis HN, "Myocardial ischemia and lipoprotein lipase activity," *Circulation* 102:1600-1601 (2000). Furthermore, in contrast to the controversy regarding hypertriglyceridemia as a risk factor for CHD in the nondiabetic population, several studies indicate that elevated triglyceride levels are independently associated with increased CHD risk in diabetic patients. Hypertriglyceridemia in diabetic patients often correlates with LDL density and subclass (*i.e.*, small, dense LDL) and decreased levels of HDL_2, which appear to increase overall risk synergistically. Havel RJ, Rapaport E, "Management of primary hyperlipidemia," *N Engl J Med* 332:1491–1498 (1995).

Characterized by increased VLDL production and impaired removal, it has been reported that patients with type 2 diabetes exhibit excessive postprandial lipemia and impaired remnant clearance. Exaggerated postprandial lipemia resulting from impaired remnant clearance is a factor in atherogenesis, involving endothelial dysfunction and enhanced oxidative stress. Karpe F, "Postprandial lipoprotein metabolism and atherosclerosis," *J Intern Med* 246:341–355 (1999); Zilversmit DB, "Atherogenesis: a postprandial phenomenon," *Circulation* 60:473–485 (1979); Patsch JR, *et al.*, "Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state," *Arterioscler Thromb* 1992;12:1336–1345 (1992); Plotnick GD, *et al.*, "Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal," *JAMA* 278:1682–1686 (1997). Postprandial lipemia consists of a heterogeneous group of triglyceride-rich particles of different composition and origin. Although 80% of the increase in postprandial triglyceride levels is accounted for by chylomicrons (which

carry a large number of triglyceride molecules), the number of endogenous (liver-derived) VLDL constitutes over 90% of the triglyceride-rich particles in the postprandial state. Delayed VLDL clearance results in the accumulation of partially catabolized VLDL remnants that are reduced in size and enriched in cholesteryl ester, and evidence indicates that these small, cholesteryl ester-enriched VLDL particles are atherogenic.

As in nondiabetic subjects, low high-density lipoprotein (HDL) levels are powerful indicators of CHD in diabetic patients. Decreased HDL levels in diabetes result from decreased production and increased catabolism of HDL and are closely related to the abnormal metabolism of triglyceride-rich lipoproteins. In insulin-resistant patients with or without overt type 2 diabetes, the composition of LDL particles is altered, resulting in a preponderance of small, triglyceride-enriched and cholesterol-depleted particles (phenotype B). A preponderance of small, dense LDL particles is related to many characteristics of insulin-resistance syndrome. In nondiabetic subjects, LDL subclass phenotype B is associated with other components of insulin-resistance syndrome, including central obesity, hypertension, glucose intolerance, and hyperinsulinemia. Selby JV, *et al.*, "LDL subclass phenotypes and the insulin resistance syndrome in women," *Circulation* 88:381–387 (1993); Reaven GM, *et al.*, "Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles," *Clin Invest* 92:141–146 (1993); Haffner SM, *et al.*, "LDL size in African Americans, Hispanics, and non-Hispanic whites: the insulin resistance atherosclerosis study," *Arterioscler Thromb Vasc Biol* 19:2234–2240 (1999).

The formation of small, dense LDL in diabetes occurs in a similar fashion to the increased formation of small and dense HDL_3. Cholesteryl ester transfer protein mediates the exchange of triglyceride from VLDL for cholesteryl ester in LDL. If sufficient LDL cholesteryl ester is replaced by triglyceride from VLDL, then when the particle comes into contact with hepatic lipase hydrolysis of newly acquired triglyceride in LDL and HDL by HTGL in turn decreases the size of LDL particles. Packard CJ, Shepherd J, "Lipoprotein heterogeneity and apolipoprotein B

metabolism," *Arterioscler Thromb Vasc Biol* 17:3542–3556 (1997). Small, dense LDL has been associated with CAD risk independently of the absolute concentrations of LDL cholesterol or other CAD risk factors. Small, dense LDL particles are more susceptible to oxidative modification. Tribble DL, *et al.*, "Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alpha-tocopherol content," *Proc Natl Acad Sci* USA 91:1183-1187 (1994). They are also particularly prone to induce endothelial dysfunction. Anderson TJ, *et al.*, "Endothelium-dependent coronary vasomotion relates to the susceptibility of LDL to oxidation in humans," *Circulation* 93:1647-1650 (1996). In addition, there is enhanced arterial wall penetration by the small LDL particles. Nielsen LB, "Transfer of low density lipoprotein into the arterial wall and risk of atherosclerosis," *Atherosclerosis* 123:1-15 (1996). The glycosylation process occurs both on the apoB and phospholipid components of LDL, resulting in profound functional alternations in LDL clearance and susceptibility to oxidative modification. Bucala R, *et al.*, "Identification of the major site of apolipoprotein B modification by advanced glycosylation end products blocking uptake by the low density lipoprotein receptor," *J Biol Chem* 270:10828–10832 (1995); Bucala R, *et al.*, "Lipid advanced glycosylation: pathway for lipid oxidation in vivo," *Proc Natl Acad Sci* USA 90:6434-6438 (1993). Clinical studies have shown an increased level of AGEs on LDL obtained from diabetics compared with healthy individuals. Bucala R, *et al.*, "Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency," *Proc Natl Acad Sci* USA 91:9441-9445 (1994). Glycosylation of LDL apoB occurs mainly on a positively charged lysine residue within the putative LDL receptor binding domain, which is essential for the recognition of LDL by the LDL receptor. *Id.* LDL glycosylation increases with glucose levels and impairs LDL receptor–mediated LDL clearance. Another atherogenic effect of glycation is to increase LDL susceptibility to oxidative modification. Advanced glycosylation of an amine-containing phospholipid component of LDL is accompanied by progressive oxidative modification of unsaturated fatty acid residues. Thus, glycation confers increased susceptibility of

LDL to oxidative modification, which is considered a critical step in its atherogenicity. Lyons TJ, "Glycation and oxidation: a role in the pathogenesis of atherosclerosis," *Am J Cardiol* 71:26B-31B (1993); Bowie A, *et al.*, "Glycosylated low density lipoprotein is more sensitive to oxidation: implications for the diabetic patient?," *Atherosclerosis* 102:63-67 (1993).

Cholesterol lowering using agents such as pravastatin have been reported to reduce the absolute risk of coronary events for diabetic patients. Goldberg RB, *et al.*, "Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the Cholesterol and Recurrent Events (CARE) trial," *Circulation* 98:2513-2519 (1998). However, the absolute clinical benefit achieved by cholesterol lowering may be greater in diabetic than in nondiabetic patients with CAD,because diabetic patients have a higher absolute risk of recurrent CAD and higher case fatality rates or because LDL cholesterol in diabetic patients is more atherogenic. Aronson D, *et al.*, "Mechanisms determining course and outcome of diabetic patients who have had acute myocardial infarction," *Ann Intern Med* 126:296-306 (1997).

The American Diabetes Association recommendations for the management of hyperlipidemia in patients with diabetes generally follow the guidelines of the National Cholesterol Education Program with several differences. American Diabetes Association. Position statement. "Management of dyslipidemia in adults with diabetes," *Diabetes Care* 21:179–182 (1998). Non-pharmacologic strategies to treat dyslipidemia in diabetics include dietary modification (similar to those recommended by the National Cholesterol Education Program), weight loss, physical exercise, and improved glycemic control. *Id.* In patients with type 1 diabetes, optimal glycemic control should result in normal or below normal lipoprotein levels and prevent the atherogenic state associated with lipoprotein glycosylation. Improved diabetic control in type 2 diabetes is beneficial but not always associated with reversal of lipoprotein abnormalities. Improved glycemic control using sulfonylurea, insulin, metformin, or thiazolidinediones can also help. The magnitude of improvement in triglycerides generally correlates with the change in glucose levels rather than the mode of therapy.

However, agents that improve insulin sensitivity such as metformin and thiazolidinediones can also lead to lower triglycerides. In addition, "perfect" glycemic control is not attained in many type 2 diabetic patients. Relatively recent publications have argued against the relevance of the traditional classification to primary and secondary CHD prevention in the setting of diabetes. Haffner SM, "Management of dyslipidemia in adults with diabetes," *Diabetes Care* 21:160-178 (1998); Haffner SM, *et al.*. "Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction," *N Engl J Med* 339:229-234 (1998). The rationale stems from both the high event rates in diabetic patients without clinical evidence of CAD (presumably because of the high rates of subclinical atherosclerosis), as well as the worse prognosis in diabetic patients who have had a clinical event compared with nondiabetic subjects. These considerations led to the suggestion that LDL cholesterol should be lowered to less than 100 mg per dL in diabetic subjects without prior CAD. *Id.*

Endothelial cells situated at the vessel wall–blood interface participate in a number of important homeostatic and cellular functions that protect from atherosclerosis and intraluminal thrombosis. Endothelial dysfunction can promote both the formation of atherosclerotic plaques and the occurrence of acute events. Endothelial dysfunction in diabetes entails profound perturbations in several critical functions of the endothelium that contribute to the initiation and progression of the atherosclerotic process, as well as to the occurrence of clinical events. It is believed that diabetes results in weakened intercellular junctions, and that AGEs diminish endothelial barrier function. The endothelial lining of the large arteries is of the continuous type characterized by tight junctions in the lateral borders, which restrict the movement of macromolecules from reaching the subendothelial space. Leukocyte adhesion to the vascular endothelium also contributes to diabetic complications. Among the earliest events in atherogenesis is the binding of mononuclear leukocytes to the endothelium with subsequent entry into the vessel wall. This is mediated through the expression of inducible adhesion molecules on the endothelial cell surface. Hyperglycemia stimulates the expression of vascular cell adhesion molecule-1 and E

selectin. In addition, AGE interaction with the AGE receptor has been reported to result in the induction of oxidative stress and, consequently, of the transcription factor NF-κB and vascular cell adhesion molecule-1. Thus, early events in the atherosclerosis process in diabetes may be mediated through enhanced adhesive interactions of monocytes with the endothelial surface. Impaired endothelium-dependent relaxation, which is mediated through the release of endothelium-derived relaxing factor (EDRF), is a consistent finding in animal models and in human diabetes and occurs in a variety of vascular beds, including the coronary arteries. Impaired endothelium-dependent relaxation has been demonstrated in both type 1 and type 2 diabetes in the absence of clinical complications, while endothelium-independent vasodilation is preserved, and impaired endothelium-dependent relaxation can be demonstrated in insulin-resistant subjects with normal glucose tolerance. De Vriese AS, *et al.*, "Endothelial dysfunction in diabetes," *Br J Pharmacol* 130:963-974 (2000).

As noted herein, hyperglycemia is recognized as the primary mediator of diabetic endothelial dysfunction. Williams SB, *et al.*, "Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo," *Circulation* 97:1695-1701 (1998). Similar to the mechanism of endothelial dysfunction observed in hypercholesterolemia, hyperglycemia-induced endothelial dysfunction is thought to result primarily from increased generation of oxygen free radicals that inactivate EDRF. Insulin resistance also contributes to endothelial dysfunction in diabetic patients. Steinberg HO, *et al.*, "Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance," *J Clin Invest* 97:2601-2610 (1996).

Diabetes is characterized by a variety of individual alterations in the coagulation and fibrinolytic systems that combine to produce a prothrombotic state. These alterations include increased platelet functional behavior, increased levels of several coagulation components, and impaired fibrinolysis. The coagulation and fibrinolytic systems are especially important in atherosclerosis because of the substantial contribution that mural thrombosis may make to the later stages of plaque progression,

and because thrombotic occlusion plays a vital role in the development of clinical events. In the vast majority of cases, the fundamental mechanism in the development of potentially life-threatening events such as unstable angina or myocardial infarction is thrombosis arising at sites of plaque disruption.

Platelet hyperaggregability, including the presence of spontaneous platelet aggregation and increased platelet aggregability induced by conventional stimuli, also increases the risk for cardiovascular events. Platelets from diabetic subjects exhibit enhanced adhesiveness and hyperaggregability. Shear-induced platelet adhesion and aggregation are also increased in diabetic patients. Knobler H, *et al.*, "Shear-induced platelet adhesion and aggregation on subendothelium are increased in diabetic patients," *Thromb Res* 90:181-190 (1998). von Willebrand factor (vWF) is involved in the initial adhesion of platelets to the subendothelium of injured vessel wall and is among the most important adhesive molecules mediating hemostatic interactions between platelets and vessel wall components. vWF is synthesized and secreted by endothelial cells, and high circulating levels of vWF are considered markers of endothelial dysfunction. In diabetic patients plasma concentrations of vWF are elevated and are closely associated with the presence of vascular complications and endothelial dysfunction. Stehouwer CD, *et al.*, "Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus," *Lancet* 340:319-323 (1992). Epidemiologic data have also demonstrated a relation between plasma vWF and insulin-resistance syndrome. Conlan MG, *et al.*, "Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study," *Thromb Haemost* 70:380-385 (1993).

A large body of evidence also indicates strong independent direct correlation between high fibrinogen plasma levels and an increased risk of CAD. Fibrinogen levels are often increased in diabetes, and this elevation is associated with poor glycemic control. Kannel WB, *et al.*, "Diabetes, fibrinogen, and risk of cardiovascular disease: the Framingham Experience," *Am Heart J* 120:672-676 (1990). The intensity of endogenous fibrinolysis depends on a dynamic equilibrium involving plasminogen

activators, primarily tissue-type plasminogen activator, and inhibitors. The principal physiologic inhibitor of tissue-type plasminogen activator is plasminogen activator inhibitor-1 (PAI-1). Attenuated fibrinolysis caused by an increase of PAI-1 activity has been associated with increased risk for myocardial infarction in patients with established CAD. Kohler HP, Grant PJ, "Plasminogen-activator inhibitor type 1 and coronary artery disease," *N Engl J Med* 342:1792-1801 (2000). Reduced plasma fibrinolytic activity caused by increased PAI-1 levels is a characteristic feature of insulin resistance and hyperinsulinemia. Elevated concentrations of PAI-1 have been recognized consistently in the plasma of hyperinsulinemic type 2 diabetics but occur also in normoglycemic insulin-resistant subjects. Juhan-Vague I, Alessi MC, "PAI-1, obesity, insulin resistance and risk of cardiovascular events," *Thromb Haemost* 78:656-660 (1997). The production of PAI-1 by adipose tissue has been demonstrated and could be an important contributor to the elevated plasma PAI-1 levels observed in insulin-resistant patients. Alessi MC, *et al.*, "Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease," Diabetes 46:860–867 (1997). Hyperglycemia can also increase PAI-1 levels because it stimulates transcription of the PAI-1 gene through an effect on its promoter region. Chen YQ, *et al.*, "Sp1 sites mediate activation of the plasminogen activator inhibitor-1 promoter by glucose in vascular smooth muscle cells," *J Biol Chem* 273:8225-8231 (1998). Although it is possible that some of the hemostatic abnormalities in diabetes are partly markers of underlying vascular disease rather than the primary abnormalities, the clotting and fibrinolytic profile of diabetic patients bears a striking similarity to that of patients at high risk for future cardiovascular events. The prothrombotic state in diabetes is said to help explain the observation that intracoronary thrombus formation is more frequently found by angioscopic examination in diabetic patients with unstable angina, and its clinical correlate, the higher risk of adverse outcome, namely death, nonfatal infarction, or recurrent unstable angina. Silva JA, *et al.*, "Unstable angina. A comparison of angioscopic findings between diabetic and nondiabetic patients," *Circulation* 92:1731–1736 (1995); Aronson D, *et al.*, "Mechanisms determining course and outcome of diabetic

patients who have had acute myocardial infarction," *Ann Intern Med* 126:296-306 (1997); Malmberg K, *et al.*, "Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndromes) Registry," *Circulation* 102:1014-1019 (2000); Calvin JE, *et al.*, "Risk stratification in unstable angina. Prospective validation of the Braunwald classification," *JAMA* 273:136–141 (1995). Thus, hyperglycemia induces a large number of alterations in vascular tissue that potentially promote accelerated atherosclerosis. Acosta J, *et al.*, "Molecular basis for a link between complement and the vascular complications of diabetes," *Proc Natl Acad Sci* USA 97:5450-5455 (2000).

Protein kinase C is also involved. The metabolic consequences of hyperglycemia can be expressed in cells in which glucose transport is largely independent of insulin. The resulting intracellular hyperglycemia has been implicated in the pathogenesis of diabetic complications through the activation of the PKC system. Ishii H, et al., "Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor," *Science* 272:728-731 (1996); Koya D, King GL, "Protein kinase C activation and the development of diabetic complications," *Diabetes* 47:859–866 (1998). High ambient glucose concentrations activate PKC by increasing the formation of diacylglycerol (DAG), the major endogenous cellular cofactor for PKC activation, from glycolytic intermediates such as dihydroxy-acetone phosphate and glyceraldehyde-3-phosphate. The elevation of DAG and subsequent activation of PKC in the vasculature can be maintained chronically. Xia P, et al., "Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase C pathway in diabetes and hypergalactosemia," *Diabetes* 43:1122-1129 (1994). PKC is a family of at least 12 isoforms of serine and threonine kinases. Although several PKC isoforms are expressed in vascular tissue, in the rat model of diabetes there is a preferential activation of PKC b2 in the aorta, heart, and retina, and PKC b1 in the glomeruli. Inoguchi T, et al., "Preferential elevation of protein kinase C isoform beta II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation," *Proc Natl Acad Sci* USA 89:11059-

11063 (1992); Koya D, et al., "Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats," *J Clin Invest* 100:115–126 (1997). The PKC system is ubiquitously distributed in cells and is involved in the transcription of several growth factors and in signal transduction in response to growth factors. In vascular smooth muscle cells, PKC activation has been reported to modulate growth rate, DNA synthesis, and growth factor receptor turnover. PKC activation increases the expression of transforming growth factor-b (TGF-b), which is one of the most important growth factors, regulating extracellular matrix production by activating gene expression of proteoglycans and collagen and decreasing the synthesis of proteolytic enzymes that degrade matrix proteins. Increased expression of TGF-b is thought to lead to thickening of capillary basement membrane, one of the early structural abnormalities observed in almost all tissues in diabetes. PKC b selective inhibitor (LY333531) attenuates glomerular expression of TGF-b and extracellular matrix proteins such as fibronectin and type IV collagen. Koya, *supra*; Koya D, et al., "Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes," *FASEB J* 14:439-447 (2000). Hyperglycemia-induced PKC activation also results in increased platelet-derived growth factor-b receptor expression on smooth muscle cells and other vascular wall cells (*e.g.*, endothelial cells, monocyte-macrophages). Inaba T, et al., "Enhanced expression of platelet-derived growth factor-beta receptor by high glucose. Involvement of platelet-derived growth factor in diabetic angiopathy," *Diabetes* 45:507–512 (1996).

Oxidative stress is widely invoked as a pathogenic mechanism for atherosclerosis. Among the sequelae of hyperglycemia, oxidative stress has been suggested as a potential mechanism for accelerated atherosclerosis. Baynes JW, Thorpe SR, "Role of oxidative stress in diabetic complications: a new perspective on an old paradigm," *Diabetes* 48:1-9 (1999). mportantly, there appears to be a strong pathogenic link between hyperglycemia-induced oxidant stress and other hyperglycemia-dependent mechanisms of vascular damage, namely AGEs formation

and PKC activation). Hyperglycemia can increase oxidative stress through several pathways. A major mechanism appears to be the hyperglycemia-induced intracellular reactive oxygen species, produced by the proton electromechanical gradient generated by the mitochondrial electron transport chain and resulting in increased production of superoxide. Nishikawa T, et al., "Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage," *Nature* 404:787-790 (2000). Two other mechanisms have been proposed that may explain how hyperglycemia causes increased reactive oxygen species formation. One mechanism involves the transition metal-catalyzed autoxidation of free glucose, as described in cell-free systems. Through this mechanism, glucose itself initiates an autoxidative reaction and free radical production yielding superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2). Wolff SP, "Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications," *Br Med Bull* 49:642–652 (1993). The other mechanism involves the transition metal-catalyzed autoxidation of protein-bound Amadori products, which yields superoxide and hydroxyl radicals and highly reactive dicarbonyl compounds. Baynes JW, Thorpe SR, "Role of oxidative stress in diabetic complications: a new perspective on an old paradigm," *Diabetes* 48:1-9 (1999). There is also evidence that hyperglycemia may compromise natural antioxidant defenses. Under normal circumstances, free radicals are rapidly eliminated by antioxidants such as reduced glutathione, vitamin C, and vitamin E. Reduced glutathione content, as well as reduced vitamin E, have been reported in diabetic patients. Yoshida K, et al., "Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux," *Diabetologia* 38:201-210 (1995); Karpen CW, et al., "Production of 12-hydroxyeicosatetraenoic acid and vitamin E status in platelets from type I human diabetic subjects," *Diabetes* 34:526-531 (1985).

As noted herein, the interaction between AGE epitopes and the cell surface AGE receptor upregulate oxidative stress response genes and release oxygen radicals. Thus, hyperglycemia simultaneously enhances both AGEs formation and oxidative stress, and the mutual facilitatory interactions between glycation and oxidation chemistry can

contribute synergistically to the formation of AGEs, oxidative stress, and diabetic complications. Indeed, there are strong correlations between levels of glycoxidation products in skin collagen and the severity of diabetic retinal, renal, and vascular disease. Beisswenger PJ, et al., "Increased collagen-linked pentosidine levels and advanced glycosylation end products in early diabetic nephropathy," *J Clin Invest* 92:212-217 (1993). Oxidative stress may also be involved in the activation of DAG-PKC in vascular tissue. Nishikawa T, et al., "Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage," *Nature* 404:787-790 (2000). Oxidants produced in the setting of hyperglycemia can activate PKC. Konishi H, et al., "Activation of protein kinase C by tyrosine phosphorylation in response to H2O2," *Proc Natl Acad Sci* USA 94:11233-11237 (1997).

The risk for CHF and idiopathic cardiomyopathy is also strongly increased in diabetes. Kannel WB, et al., "Role of diabetes in congestive heart failure: the Framingham study," *Am J Cardiol* 34:29-34 (1974); Shindler DM, et al., "Diabetes mellitus, a predictor of morbidity and mortality in the Studies of Left Ventricular Dysfunction (SOLVD) Trials and Registry," *Am J Cardiol* 77:1017-1020 (1996); Ho KK, et al., "The epidemiology of heart failure: the Framingham Study," *J Am Coll Cardiol* 22:6A-13A (1993). Although data on the effect of diabetes on the prognosis of patients with CHF are limited, several studies suggest that diabetes is an independent predictor of poor prognosis in this setting. In the Studies of Left Ventricular Dysfunction study, diabetes was an independent predictor of morbidity and mortality in patients with symptomatic heart failure, asymptomatic patients with an ejection fraction less than or equal to 35%, and in the registry population. Shindler, *supra*. One reason for the poor prognosis in patients with both diabetes and ischemic heart disease seems to be an enhanced myocardial dysfunction leading to accelerated heart failure. Grundy SM, et al., "Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association," *Circulation* 100:1134-1146 (1999).

The cardiomyopathic process associated with diabetes mellitus manifests initially as diminished left ventricular compliance in the presence of normal left ventricular

systolic function. Zarich SW, et al., "Diastolic abnormalities in young asymptomatic diabetic patients assessed by pulsed Doppler echocardiography," *J Am Coll Cardiol* 12:114-120 (1988); Paillole C, et al., "Prevalence and significance of left ventricular filling abnormalities determined by Doppler echocardiography in young type I (insulin-dependent) diabetic patients," *Am J Cardiol* 64:1010-1016 (1989); Mildenberger RR, et al., "Clinically unrecognized ventricular dysfunction in young diabetic patients," *J Am Coll Cardiol* 4:234--238 (1984). Diastolic abnormalities occur in 27% to 69% of asymptomatic diabetic patients. A lower ejection fraction in response to dynamic exercise in the presence of a normal resting ejection fraction has been demonstrated in several studies, indicating that contractile reserve is decreased in many asymptomatic patients with diabetes. Mildenberger, *supra*; Shapiro LM, et al., "Left ventricular function in diabetes mellitus. II: Relation between clinical features and left ventricular function," *Br Heart J* 45:129-132 (1981); Mustonen JN, et al., "Left ventricular systolic function in middle-aged patients with diabetes mellitus," *Am J Cardiol* 73:1202-1208 (1994). Systolic dysfunction may appear, usually in patients with long-standing disease who suffer from advanced microvascular complications. Raev DC, "Which left ventricular function is impaired earlier in the evolution of diabetic cardiomyopathy? An echocardiographic study of young type I diabetic patients," *Diabetes Care* 17:633-639 (1994). However, even subclinical cardiomyopathy with reduced myocardial reserve may become clinically important in the presence of myocardial ischemia or with coexistent uncontrolled hypertension. Stone PH, et al., "The effect of diabetes mellitus on prognosis and serial left ventricular function after acute myocardial infarction: contribution of both coronary disease and diastolic left ventricular dysfunction to the adverse prognosis. The MILIS Study Group," *J Am Coll Cardiol* 14:49-57 (1989).

It is also understood that the coexistence of hypertension and diabetes exerts a particularly deleterious effect on the heart. The coexistence of hypertension has been considered a major factor in the expression of diastolic dysfunction in diabetic patients. Grossman E, Messerli FH, "Diabetic and hypertensive heart disease," *Ann Intern Med* 125:304-310 (1996). In hypertensive subjects, diabetes is an important

precursor of CHF, with a greater relative risk in women than in men. Levy D, et al., "The progression from hypertension to congestive heart failure," *JAMA* 275:1557-1562 (1996). The mechanisms responsible for the increased risk for the development of CHF are not fully understood, but may be related in part to an exaggerated increase in left ventricular mass. Grossman E, et al., "Left ventricular mass in diabetes-hypertension," *Arch Intern Med* 152:1001-1004 (1992).

Obesity, which is characterized by insulin resistance and hyperinsulinemia, is also strongly correlated with increased left ventricular mass independent of age and blood pressure. Lauer MS, et al., "The impact of obesity on left ventricular mass and geometry. The Framingham Heart Study," *JAMA* 266:231-236 (1991). Furthermore, left ventricular mass in normotensive obese subjects is related more to the severity of insulin resistance than to the obesity itself as expressed by the body mass index. Sasson Z, et al., "Insulin resistance is an important determinant of left ventricular mass in the obese," *Circulation* 88:1431-1436 (1993).

In hypertensive patients with normal glucose tolerance, who commonly exhibit insulin resistance and hyperinsulinemia, left ventricular mass has been shown to correlate with the degree of insulin resistance. Ohya Y, et al., "Hyperinsulinemia and left ventricular geometry in a work-site population in Japan," *Hypertension* 27:729-734 (1996); Verdecchia P, et al., "Circulating insulin and insulin growth factor-1 are independent determinants of left ventricular mass and geometry in essential hypertension," *Circulation* 100:1802-1807 (1999). A similar association is also observed in nonhypertensive insulin-resistant subjects. Marcus R, et al., "Sex-specific determinants of increased left ventricular mass in the Tecumseh Blood Pressure Study," *Circulation* 90:928-936 (1994).

Hypertension, insulin resistance, hyperinsulinemia, and type 2 diabetes are commonly associated and result in a high risk for cardiovascular complications. Reaven GM, Laws A, "Insulin resistance, compensatory hyperinsulinaemia, and coronary heart disease," *Diabetologia* 37:948-952 (1994); Agewall S, et al., "Carotid artery wall intima-media thickness is associated with insulin-mediated glucose disposal in men at high and low coronary risk," *Stroke* 26:956-960 (1995). Left

ventricular mass is a strong predictor of cardiac and cerebrovascular morbidity independent of blood pressure or other risk factors, as well as a powerful risk factor for the development of symptomatic CHF, and it is believed that the association between insulin resistance and left ventricular hypertrophy may contribute to the increase risk of symptomatic CAD in insulin-resistant subjects.

Several reports have focussed on metabolic abnormalities including abnormal intracellular Ca^{2+} handling, defects in myocardial glucose use, and activation of PKC as possible explanations for the pathogenesis of diabetic cardiomyopathy. Additionally, there may also be a role for AGEs in the pathogenesis of diabetic cardiomyopathy. Diabetic patients have increased arterial stiffness compared with nondiabetic individuals and manifest diminished left ventricular compliance at a young age. Several investigators have demonstrated that diabetes has several features of accelerated aging at the tissue level and at the level of collagen itself. Aging and diabetes mellitus are associated with cross-linking and nonenzymatic glycosylation of collagen. This led to the concept that glycosylation could explain the progressive cross-linking of collagen during normal aging and at an accelerated rate in diabetes, leading to changes in vascular tissue mechanical properties. Thus, disturbances of vascular and cardiac mechanical properties in diabetes may be caused by a common mechanism. Among the structural alterations associated with AGEs formation is collagen-to-collagen cross-linking, which alters the structure and function of this protein, leading to tissue rigidity. Increased arterial stiffness in patients with diabetes strongly correlates with increased aorta and myocardial collagen advanced glycation. Airaksinen KE, et al., "Diminished arterial elasticity in diabetes: association with fluorescent advanced glycosylation end products in collagen," *Cardiovasc Res* 27:942-945 (1993). Further evidence supporting the AGE hypothesis is the observation that agents that specifically inhibit AGE formation effectively prevent the pathologic stiffening process of diabetes and aging. Norton GR, et al., "Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats," *Circulation* 93:1905–1912 (1996); Huijberts MS, et al., "Aminoguanidine treatment increases elasticity and decreases fluid filtration of large

arteries from diabetic rats," *J Clin Invest* 92:1407–1411 (1993). For example, treatment of diabetic rats with aminoguanidine, an inhibitor of AGE formation, reportedly increased carotid artery compliance, decreases aortic impedance, and prevented the decreased myocardial compliance. *Id.*

It has been attempted with greater or lesser efficacy to pharmacologically influence the process of nonenzymatic glycation and AGE products formation using, in general, two approaches. The first is inhibition of the rearrangement from early to advanced glycation endproducts by means of hydrasine:aminoguanidine hydrochloride or analogue. The second is the breaking of already existing AGE products with substituted thiazolium salts. Pharmacologic activity of aminoguanidine may render impossible or retard some of microvascular complications in animal model. Although the mechanism of aminoguanidine action has not been completely understood, it may inhibit some stages in a series of chemical reactions leading to glycation end-product formation. In spite of the first encouraging results, clinical trials of aminoguanidine in patients with type 2 diabetes mellitus have been suspended due to adverse effects. See, for example, Brownlee M., "Negative consequences of glycation," *Metabolism* 2000; 49(suppl 1): 9-13; Singh R, Barden A, Mori T, Beilin L., "Advanced glycation end-products: a review," *Diabetologia* 2001; 44: 129-146; Vlassara H, Bucala R, Striker L., "Pathogenic effects of AGEs: Biochemical, biologic, and clinical implications for diabetes and aging," *Lab Invest* 1994;70:138-151; Lyons T, Jenkins AJ., "Glycation, oxidation and lipoxidation in the development of the complications of diabetes mellitus: a 'carbonyl stress' hypothesis," *Diabetes Rev* 1997;5:365-391.

As indicated herein, it is understood that diabetes mellitus is a major source of morbidity in developed countries. Among its co-morbid conditions, atherosclerosis is perhaps the most important. Since the availability of insulin, up to three-quarters of all deaths among diabetics can be directly attributed to CAD. In patients with type 1 diabetes, up to one third will die of CAD by the age of 50 years. A number of known risk factors for CAD, such as hypertension, central obesity and dyslipidemia, are more common in diabetics than in the general population. Thus diabetes represents a major contributing factor to the CAD burden in the developed world, and most of the excess

attributed risk of CAD in diabetics cannot be readily quantified with the use of traditional risk factors analysis. As indicated, the relation between hyperglycemia and CAD is the subject of debate because serum glucose does not consistently predict the existence of CAD. However, recent prospective data have clearly established a link between a marker for chronic average glucose levels (HbA1c) and cardiovascular morbidity and mortality. There are established sequelae of hyperglycemia, such as cytotoxicity, increased extracellular matrix production and vascular dysfunction and all have been implicated in the pathogenesis of diabetes-induced vascular disease, and the formation of AGEs correlate directly with the vascular and renal complications of diabetes mellitus.

Patients with diabetes mellitus are particularly susceptible to morbidity and mortality resulting from cardiovascular diseases, especially atherosclerosis. therosclerosis is a disease of the walls of the aorta and large arteries, thought to be initiated by injury to the intimal layer of cells that line the lumen of the blood vessel. Progression of the disease is characterized by infiltration of lipids into the vessel wall and the formation of fibrous tissue called the atheromatous plaque. Clinical symptoms of atherosclerosis do not usually occur until over half of the lumen becomes obstructed (occluded) by the plaque, typically in the fifth and sixth decades of life. Consequently, studies on the role of plasma lipids in health and in the genesis of CHD have dominated research on CHD over the past several decades. Current positive evidence documents the premise that the following are important risk factors: family history, a high plasma concentration of low-density lipoprotein (LDL) and a low concentration high density lipoprotein (HDL) cholesterol (separately as well as jointly), high plasma concentration of apoB (the major protein fraction of the LDL particle), high plasma lipoprotein (a) (Lp(a)) concentration, high plasma fibrinogen concentration, hypertension, diabetes, obesity, increased plasma concentration of homocysteine (all these themselves have genetic determinants), high dietary fat intake, lack of exercise, stress, and smoking.

The pathogenesis of the atherosclerosis in diabetes mellitus is not entirely clear and conventional risk factors such as smoking, obesity, blood pressure and serum

lipids fail to explain fully this excess risk. Important features in the pathogenesis of atherosclerosis appear to include vascular endothelial injury, platelet adhesion and activation, fibrin deposition, cellular proliferation, and low-density lipoprotein cholesterol accumulation. Fibrin deposition is an invariable feature in atherosclerotic lesions. Therefore, disturbances of haemostasis leading to accelerated fibrin formation (hypercoagulability) and delayed fibrin removal (impaired fibrinolysis) may contribute to the development of atherosclerosis. Hyperactive platelets, hypercoagulability and impaired fibrinolysis also promote thrombosis formation at the site of ruptured atherosclerotic lesion and lead to final occlusion event in the progression of atherosclerosis.

Although platelet counts are normal in patients with diabetes mellitus, multiple studies offer evidence of enhanced activation or increased platelet activity. Additionally, an increase in plasma levels of von Willebrand factor (vWF), which is important for the adhesion of platelets to subendothelial structures, has been reported in diabetic patients. Hyperactive platelets may form microaggregates leading to capillary microembolization. In patients with diabetes the resulting relative tissue hypoxia may in the long-term precede clinically detectable microangiopathy. It has been speculated that microembolization of the vasa vasorum of the large vessels by hyperactive platelets may also be the initial event in the development of atherosclerosis. Secretion of mitogenic, oxidative or vasoconstrictive substances by platelets activated in response to endothelial injury amplifies and accelerates the progression of atherosclerosis. Acute thrombotic events in the arterial circulation are also triggered by platelets.

In diabetes mellitus disturbances of haemostasis leading to hypercoagulability have been observed in numerous studies. Besides altered screening tests, alterations of several coagulation factors and inhibitors have been occasionally described. A problem encountered when studying the association between hypercoagulability and atherosclerosis is the number of laboratory tests proposed to detect hypercoagulability and the wide variability of such tests in a given subject. Results of cohort studies have shown that among different coagulation factors analyzed, increased concentration of

fibrinogen, factor VII and vWF have predictive value for coronary atherosclerosis and can be considered as risk factors for cardiovascular events. Increase in these factors could participate in the pathogenesis of atherosclerosis, predominantly of coronary arteries.

Fibrinogen is a parameter that has been studied most extensively in epidemiological studies. A relationship has been established between plasma concentration of fibrinogen, the quantity of fibrinogen and fibrin present in the vessel wall and the severity of atherosclerosis. These associations are more pronounced in diabetic patients. High fibrinogen concentration is observed also in diabetic patients, especially in those with albuminuria. Relationship between fibrinogen and insulin resistance is controversial. Free fatty acids have been suggested to explain the fibrinogen-insulin resistance relationship, because a simultaneous increase in free fatty acids and fibrinogen is seen in variety of clinical and experimental condition. This relationship might also result from an inflammatory reaction accompanying atherosclerosis.

Factor VII is a vitamin K dependent protein synthesized in the liver. It is the key enzyme in the initiation of blood coagulation. The Northwick Park Heart Study and the PROCAM study have shown that there is a positive correlation between increased factor VII and cardiovascular mortality. Plasma concentration of factor VII is closely related to several environmental factors, mainly triglycerides and cholesterol levels. These associations are highly dependent on dietary intake. An increase in factor VII has been described in diabetes mellitus and is more pronounced in those with microalbuminuria. Only limited data are available concerning the contributory role of insulin resistance to elevated factor VII. The relationship between factor VII and insulin and proinsulin have been described as very weak or present only in women. Factor VII which is influenced by the efficiency of the metabolism of triglyceride-rich lipoproteins could in this way be modified in insulin resistance.

Increased plasma concentration of vWF has been shown to be predictive of re-infarction and mortality in survivors of myocardial infarction, of cardiac events in healthy people and in patients with angina pectoris. The European Concerted Action

on Thrombosis study showed that vWF predictability was not affected by the adjustment with other classical coronary risk factors such as body mass index, lipid disorders or smoking. As vWF levels are dependent on the acute phase reaction like fibrinogen, and vWF correlates positively with fibrinogen or C-reactive protein levels, it has to be evaluated if vWF is a risk factor irrespective of fibrinogen level. In type 2 diabetic patients vWF levels are higher in microalbuminuric patients. vWF is very poorly or not at all related to insulin resistance.

Hypercoagulability can be judged also from increased levels of markers of coagulation system activation, which reflect enhanced thrombin generation. Prothrombin fragment 1+2 released when thrombin is formed from prothrombin is increased in diabetes. Once activated, thrombin is rapidly inactivated by antithrombin, forming thrombin-antithrombin complexes, which subsequently circulate and are removed by the liver. Multiple studies have documented elevated thrombin-antithrombin complexes in diabetes. Fibrinopeptide A is released when fibrinogen is converted to fibrin by thrombin. Thus, fibrinopeptide A levels are increased during coagulation. Measurement of fibrinopeptide A in diabetes has yielded a variety of results, from elevated to normal.

The fibrinolytic system is natural defence against thrombosis. A balance exists between plasminogen activators and inhibitors, and impairment of this balance can be caused either by diminished release of tissue plasminogen activator (t-PA) or increased levels of plasminogen activator inhibitor 1 (PAI-1). PAI-1 is a serine protease inhibitor and evidence suggests that it is the major regulator of the fibrinolytic system. It binds and rapidly inhibits both single- and two-chain t-PA and urokinase. t-PA and PAI-1 rapidly form an inactive irreversible complex.

Abnormalities of the fibrinolytic system have been described in both type 1 and type 2 diabetes. Impaired fibrinolysis, as described in diabetes type 2, is commonly accompanied by an increased plasma levels of PAI-1 and by increased concentration of t-PA antigen, which reflects predominantly t-PA/PAI-1 complexes. In type 1 diabetes results are mixed, and diminished, normal and enhanced fibrinolysis have all been reported. In subjects with type 2 diabetes a variety of risk factors are

independently associated with impaired fibrinolysis: obesity, hypertension, dyslipidaemia, glucose intolerance, hyperinsulinaemia and insulin resistance. These factors often tend to converge and numerous studies have attempted to dissect out the independent contribution of the above risk factors in determining fibrinolytic activity in diabetes, but this task has been hampered by the complex relationship between them. In non-diabetic subjects, insulin resistance is paralleled by increased insulin and both correlate with triglyceride levels. Thus any one or more of these variables may explain interrelationship with PAI-1. By contrast in type 2 diabetes, insulin resistance, insulin concentration and triglyceride levels are less tightly interdependent in explaining increased PAI-1.

Impaired fibrinolysis not only predisposes to thrombotic events but also plays a role in the formation and progression of atherosclerotic lesions. Increased synthesis of PAI-1 has been demonstrated in atherosclerotic lesions. This may lead to fibrin deposition during lesion rupture, contributing to the progression of the lesion. PAI-1 within the lesion inhibits plasmin formation, which plays an important role in cleaving extracellular matrix proteins, directly or via activation of metalloproteinases. This may lead to stabilization and further growth of atherosclerotic lesion.

Changes in the fibrinolytic system also play an important role in microangiopathy. Urokinase and plasmin are activators of latent metalloproteinases, such as collagenases, that are responsible for proteolysis of extracellular matrix proteins. Increased PAI-1 may lead to basement membrane thickening observed in microangiopathy.

Hyperinsulinaemia has also been associated with cardiovascular disease in non-diabetic subjects. In those with type 2 diabetes the extent of hyperinsulinemia parallels plasma PAI-1 activity, and insulin has been implicated as a major physiological regulator of PAI-1. Despite population correlations of insulin and PAI-1, and the effect of insulin on PAI-1 production in vitro, a direct effect of insulin on PAI-1 levels in vivo in humans has not been shown, either with intravenous infusion of insulin or by an oral glucose load with the aim of producing portal hyperinsulinemia. Thus, in humans there is little evidence that interventions resulting

in increased concentration of insulin in vivo increase PAI-1. On the other hand reducing insulin levels and insulin resistance by exercise, weight loss and the drug metformin has been shown to reduce PAI-1. In patients with type 2 diabetes approximately 30 % of fasting immunoreactive insulin concentration consists of proinsulin-like molecules. The elevated levels of PAI-1 in these subjects may, therefore, be a consequence of precursor insulin rather than insulin itself.

Hyperglycemia is an additional risk factor for impaired fibrinolysis. Glucose can directly increase PAI-1 production in human endothelial cells. In patients with type 2 diabetes a significant correlation between glucose concentration and PAI-1 and has been observed. It has been proposed that insulin resistance or hyperinsulinemia could influence the synthesis of PAI-1 via effects on lipid metabolism. In patients with diabetes, dyslipidaemia, in particular high triglyceride and low high-density lipoprotein level, is common. Studies *in vitro* have reportedly demonstrated the effect of various lipoproteins on PAI-1 synthesis. Very-low-density lipoproteins from hypertriglyceridaemic patients increase endothelial cell production of PAI-1 to a greater degree than that from normo-triglyceridaemic subjects. Oxidized low-density lipoproteins also stimulate endothelial cell PAI-1 synthesis as does lipoprotein(a). Lipoprotein(a), low-density lipoprotein, and high-density lipoproteins also suppress t-PA secretion from human endothelial cells in dose dependent manner.

In sum, there is significant laboratory evidence of chronic platelet activation, enhanced coagulation and impaired fibrinolysis in patients with diabetes mellitus. These disturbances of haemostasis favor development of atherosclerosis and thrombosis in particularly of coronary arteries.

Metals are present naturally in body and many are essential for cells (*e.g.*, Cu, Fe, Mn, Ni, Zn). However, all metals are toxic at higher concentrations. One reason metals may become toxic is because they may cause oxidative stress, particularly redox active transition metals, which can take up or give off an electron (*e.g.*, Fe2+/3+, Cu+/2+) can give rise to free radicals that cause damage (Jones *et al.*, "Evidence for the generation of hydroxyl radicals from a chromium(V) intermediate isolated from the reaction of chromate with glutathione," *Biochim. Biophys. Acta* **286**: 652–655

(1991); Li, Y. and Trush, M.A. 1993. DNA damage resulting from the oxidation of hydroquinone by copper: role for a Cu(II)/Cu(I) redox cycle and reactive oxygen generation," *Carcinogenes* 7: 1303–1311 (1993). Another reason why metals may be toxic is because they can replace other essential metals in or enzymes, disrupting the function of these molecules. Some metal ions (*e.g.*, Hg+ and Cu+) are very reactive to thiol groups and can interfere with protein structure and function.

As noted herein, humans subject to type 2 diabetes or abnormalities of glucose mechanism are particularly at risk to the precursors of heart failure, heart failure itself and a miscellany of other diseases of the arterial tree. It has been reported that in Western countries, more than 50% of patients with type 2 diabetes die from the effects of cardiovascular disease. See, Stamler *et al.*, *Diabetes Care* 16:434-44 (1993). It has also been reported that even lesser degrees of glucose intolerance defined by a glucose tolerance test (impaired glucose tolerance, or "IGT") still carry an increased risk of sudden death. See, Balkau *et al.*, *Lancet* 354;1968-9 (1999). For a long time, it was assumed that this reflected an increased incidence of coronary atherosclerosis and myocardial infarction in diabetic subjects. However, evidence is mounting that diabetes can cause a specific heart failure or cardiomyopathy in the absence of atherosclerotic coronary artery disease.

Cardiac function is commonly assessed by measuring the ejection fraction. A normal left ventricle ejects at least 50% of its end-diastolic volume each beat. A patient with systolic heart failure commonly has a left ventricular ejection fraction less than 30% with a compensatory increase in end-diastolic volume. Hemodynamic studies conducted on diabetic subjects without overt congestive heart failure have observed normal left ventricular systolic function (LV ejection fraction) but abnormal diastolic function suggesting impaired left ventricular relaxation or filling. See, Regan *et al.*, *J. Clin. Invest.* 60;885-99 (1977). In a recent study, 60% of men with type 2 diabetes without clinically detectable heart disease were reported to have abnormalities of diastolic filling as assessed by echocardiography. See, Poirier *et al.*, *Diabetes Care* 24;5-10 (2001). Diagnosis may be made, for example, by noninvasive measurements. In the absence of mitral stenosis, mitral diastolic blood flow measured by Doppler

echocardiography is a direct measure of left ventricular filling. The most commonly used measurement is the A/E ratio. Normal early diastolic filling is rapid and is characterized by an E-wave velocity of around 1m/sec. Late diastolic filling due to atrial contraction is only a minor component, and the A-wave velocity is perhaps around 0.5m/sec. This gives a normal A/E ratio of approximately 0.5. With diastolic dysfunction, early diastolic filling is impaired, atrial contraction increases to compensate, and the A/E ratio increases to more than 2.0.

Treatment of diabetic cardiomyopathy is difficult and the options are limited. Tight control of blood glucose levels might prevent or reverse myocardial failure, although this may be true only in the early stages of ventricular failure. Angiotensin converting enzyme inhibitors such as captopril improve survival in heart failure particularly in patients with severe systolic heart failure and the lowest ejection fractions. There are, however, various therapies for diabetic cardiomyopathy that are not recommended. For example, inotropic drugs are designed to improve the contraction of the failing heart. However, a heart with pure diastolic dysfunction is already contracting normally and it is believed that inotropic drugs will increase the risk of arrhythmias. Additionally, there appears to be no logical reason to use vasodilator drugs that reduce afterload and improve the emptying of the ventricle because ejection fraction and end-diastolic volume are already normal. Afterload reduction may even worsen cardiac function by creating a degree of outflow obstruction.

Diuretics are the mainstay of therapy for heart failure by controlling salt and water retention and reducing filling pressures. However, they are contraindicated in diastolic dysfunction where compromised cardiac pump function is dependent on high filling pressures to maintain cardiac output. Venodilator drugs such as the nitrates, which are very effective in the management of systolic heart failure by reducing pre-load and filling pressures, are understood to be poorly tolerated by patients with diastolic heart failure. Ejection fraction and end-systolic volume are often normal and any reduction in pre-load leads to a marked fall in cardiac output. Finally, there is concern about the use of β-blockers in heart failure because of their potential to

worsen pump function. There is also concern regarding the administration of β-blockers to patients with diabetes who are treated with sulphonylurea drugs and insulin due to a heightened risk of severe hypoglycaemia.

Thus, it will be understood that the mechanisms underlying the long-term complications of diabetes, including associated heart diseases and conditions, are complex and have long been studied without the discovery of clear, safe and effective therapeutic interventions. There is a need for such therapies, which are described herein.

We believe without wishing to be bound that the reactive oxygen species superoxide is generated in excess in poorly controlled diabetes by over-expression of the enzyme *fructosamine oxidase* (see our PCT/NZ99/00161 (WO 00/18392)). In the presence of excess free iron, or a combination of excess free copper and excess free iron, such superoxide anions we believe without wishing to be bound are rapidly degraded to form hydroxyl radicals via an iron catalysed Haber-Weiss reaction. See, Halliwell B & Gutteridge JMC "Free radicals in Biology and Medicine" Clarendon Press, Oxford pp. 136-76 (1989). Hydroxyl radicals are extremely reactive species and could cause the damage to basement membrane proteins and histopathological changes that are typical of diabetic microvascular disease. See, Robbins SL, Cotran RS, Kumar V. "Pathologic basis of disease" 3rd ed WB Saunders, pp. 991-1061. (1984)).

The heart is the most susceptible of all the body organs to premature aging and free radical oxidative stress. In our own studies (using the streptozocin-diabetic (STZ) rat model), we have found a high frequency of cardiomyopathy and macrovascular disease in severely diabetic animals. Iron concentrations were found to be increased in the heart tissue and consistent changes were present in the walls of major blood vessels of such diabetic animals over iron concentrations found in corresponding tissue of non diabetic animals and/or animals not exhibiting cardiomyopathy or macrovascular disease.

Treatment with iron chelators we have found both (i) reduces iron concentrations in heart tissue and the walls of major blood vessel and (ii) ameliorates many of the long-term cardiac sequelae. Morbidity and mortality were reduced.

Iron is an essential element for normal cellular function and general health. However, iron does play a pathologic role in the development of cardiomyopathy in the iron overload states of hemochromatosis, beta-thalassemia, and transfusion siderosis. Iron accumulation in the heart may be 10 to 15 times normal, caused by the wasteful, ineffective erythropoiesis of an enormously expanded erythroid marrow. Removal of iron via phlebotomy for hemochromatosis and chelation therapy for beta-thalassemia are proven treatments. Removal of iron via phlebotomy has recently been reported to increase in the short term the vasodilation induced by glyceryl trinitrate in men with high-ferritin Type II Diabetes. See Fernandez-Real et. Al (Diabetes Care 25i 2249-55 (2002).

Our hypothesis described hereafter in more detail (to which we do not wish to be bound) is that diabetic cardiomyopathy is a common, diagnosable, and potentially treatable condition. Pathologically it is due to a degree of iron overload in the heart (up to 5 times normal and more usually about 1.2 to about 3 times normal) and to a direct free iron effect on the myocytes. This implies that the disease process is reversible if the tissue iron concentration can be controlled. The advent of Doppler echocardiography and magnetic resonance imaging can identify the population at risk. Treatment with specific iron chelators will benefit an ever increasing number and spectrum of the population provided that iron deficiency anemia and subclinical iron deficiency states are excluded.

The diagnosis of uncomplicated iron deficiency anemia can be made using hematological measures in combination with standard biochemical iron studies. The standard biochemical iron tests are serum iron, serum TIBC (total iron binding capacity), iron saturation, and serum ferritin. However, these tests do not readily identify the population with subclinical iron deficiency. There are three tests which have been shown to detect the stage of iron deficiency after iron stores have been depleted, but before anemia develops:

1. **Zinc protoporphyrin** has been used as a screening test for iron deficiency in children for many years. It is synthesized when there is not enough iron available for normal hemoglobin synthesis. Although not as sensitive as ferritin, it is not affected by acute or chronic inflammatory conditions which may lead to an elevated ferritin level even when iron stores are depleted. Zinc protoporphyrin is determined by measuring the fluorescence of a sample of hemolysed red cells.

2. **Serum transferrin receptors** correlate directly with erythropoietic activity and inversely with the iron available for erythropoiesis. Serum transferrin receptor mass increases at the onset of iron deficiency erythropoiesis, but not in anemia caused by chronic disease. The analysis of serum transferrin receptors can be carried out using standard automated equipment.

3. **Reticulocyte hemoglobin content** acts as an indicator of iron restricted erythropoiesis. It is a strong predictor of iron deficiency and may be measured on routine automated hematology analysers.

STATEMENT OF INVENTION

It is an object of the present invention to provide methods of treatment and related methods, uses and pharmaceutical compositions that ameliorate, prevent and/or treat (e.g. by tissue repair) any one or more disease states of the cardiovascular tree (including the heart) and dependent organs (eg; retina, kidney, nerves, etc.) exacerbated by non-intracellar free iron values levels.

It is also an object of the present invention to provide methods of treatment and related methods, uses and pharmaceutical compositions that have a potential to ameliorate, prevent or treat diabetic cardiomyopathy and diabetic macrovascular disease.

Reference herein to diseases of the cardiovascular tree and diseases of dependent organs includes any one or more of

(i) **disorders of the heart muscle** (cardiomyopathy or myocarditis) such as idiopathic cardiomyopathy, metabolic cardiomyopathy which includes diabetic cardiomyopathy, alcoholic cardiomyopathy, drug-induced

cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy,

or

(ii) atheromatous disorders of the major blood vessels (**macrovascular disease**)

such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries,

or

(iii) toxic, drug-induced, and metabolic (including hypertensive and/or diabetic disorders of small blood vessels (**microvascular disease**) such as the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems,

or

(iv) **plaque rupture of atheromatous lesions of major blood vessels** such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries and the popliteal arteries.

The present invention relates to any such ailments and their treatment irrespective (unless otherwise stated) of any diabetic and/or glucose abnormality state of the mammalian patient. Accordingly included within the categories of disease of patients that might usefully be targeted by the procedures of the present invention are any one or more of the following non exhaustive list:

diabetic cardiomyopathy,

diabetic acute coronary syndrome (eg; myocardial infarction – MI),

diabetic hypertensive cardiomyopathy,

acute coronary syndrome associated with impaired glucose tolerance (IGT),

acute coronary syndrome associated with impaired fasting glucose (IFG),

hypertensive cardiomyopathy associated with IGT, hypertensive cardiomyopathy associated with IFG,

ischaemic cardiomyopathy associated with IGT, ischaemic cardiomyopathy associated with IFG,

ischaemic cardiomyopathy associated with coronary heart disease (CHD),

acute coronary syndrome not associated with any abnormality of the glucose metabolism,

hypertensive cardiomyopathy not associated with any abnormality of the glucose metabolism,

ischaemic cardiomyopathy not associated with any abnormality of the glucose metabolism (irrespective of whether or not such ischaemic cardiomyopathy is associated with coronary heart disease or not), and

any one or more disease of the vascular tree including, by way of example, disease states of the aorta, carotid, cerebrovascular, coronary, renal, retinal, vasa nervorum, iliac, femoral, popliteal, arteriolar tree and capillary bed.

We believe without wishing to be bound that in the aforementioned diabetic states or glucose metabolism abnormal states that diabetic complications in the distal regions of the arterial tree can be mediated by the regimen of the present invention whilst at the same time improving more proximal conditions.

With a non diabetic patient the complications arising from iron values content of the whole body are more proximal than distal but nonetheless mediation of and/or repair of such damage (including of or to the aorta, carotid, cerebrovascular, coronary, renal, retinal, vasa nervorum, iliac, femoral, popliteal, arteriolar tree and capillary bed) we believe is improved by the regimen of the present invention.

As used herein "values" includes any form of the metal in so far as its oxidation state is concerned provided such values can be scavenged or chelated by the therapy proposed.

As used herein the term "comprises" where not associated with "includes" means has, is or includes.

As used herein the term "and/or" means both "and" and "or".

As used herein the addition of "(s)" as part of a word embraced both the singular and plural of that word.

As used herein the term "diabetic" refers to a human being or other mammal suffering from Type II diabetes or impaired glucose tolerance (IGT).

As used herein the term "diabetic" in respect of cardiomyopathy (or the tendency thereto) and in respect of macrovascular disease (or the tendency thereto) of a mammal refers to a human being suffering from Type II diabetes or impaired glucose tolerance (IGT).

The term "cardiomyopathy" as used herein and where the context so allows includes both cardiomyopathy and associated heart failure.

The term "macrovascular disease" as used herein refers to vascular disease such as atherosclerosis in blood vessels such as large elastic arteries, and large and intermediate muscular arteries in particular, but may also include intermediate and small arteries as well as most veins (Stebhens, 1995) although it does not include blood vessels such as arterioles and capillaries.

As used herein the terms "subjecting the patient" or "administering to" includes any active or passive mode of ensuring the *in vivo* presence of the active compound(s) or metabolite(s) irrespective of whether one or more dosage to the mammal, patient or person is involved. Preferably the mode of administration is oral although other modes (particularly parenteral eg; intravenous, intra muscular, etc.) are also contemplated.

As used herein, "therapeutically effective amount" refers to a predetermined amount of an agent calculated to achieve a desired therapeutic effect.

As used herein, "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" refers to a carrier that does not cause an adverse physical reaction upon administration and one in or from which a therapeutic agent is sufficiently carried or can leave to deliver a therapeutically effective amount.

As used herein, "mammal" has its usual meaning and includes primates (e.g.; humans and nonhumans primates), experimental animals (e.g.; rodents such as mice and rats), farm animals (such as cows, hogs, sheep and horses), and domestic animals (such as dogs and cats).

The term "elevated" has the meaning

As used herein, the terms "treatment" or "treating" of a condition and/or a disease in a mammal, means, where the context allows,

(i) preventing the condition or disease, that is, avoiding any clinical symptoms of the disease;

(ii) inhibiting the condition or disease, that is, arresting the development or progression of clinical symptoms; and/or

(iii) relieving the condition or disease, that is, causing the regression of clinical symptoms.

As used herein "associated with" simply means both circumstances exist and should not be interpreted as meaning one necessarily is causally linked to the other.

The term "chelatable iron" includes iron in any of its chelatable forms including different oxygen states. Accordingly the term "iron" [eg; could be elemental, salts, etc.] means iron in any appropriate form in the body available for such chelation (ie; in extracellular tissue [eg; possibly bound to cell exteriors and/or collagen as opposed to intracellular tissue]) and/or capable of being reduced by other means (eg; zinc administration).

The term "chelatable iron" whether in respect of heart tissue or the walls of major blood vessels also refers to iron or salts of iron and particularly, although not solely, to those forms of iron directly or indirectly (e.g. in conjunction with copper) capable of catalysis of the Haber-Weiss Reaction.

As used herein the term "Haber-Weiss Reaction" refers to that reaction described hereinafter as the third stage of the hypothesis embodied in Figure 2 appended hereto.

Some preferred chelators of iron values appropriate for mammalian administration include (where appropriate as a salt such as a suitable calcium sodium salt to avoid hypocalcemia):

trientine (triene), [more a copper chelator]

ethylenediaminetetraacetic acid (EDTA),

diethylenetriaminetetraacetic acid (DPTA),

2,2,2 tetramine tetrahydrochloride (TETA),

2,3,2 tetramine tetrahydrochloride,

D-penicillamine (DPA)

1,4,8,11 tetraazacyclotretradecane (Cyclam),

5,7,7',12,14,14' hexamethyl-1,4,8,11 tetraazacyclotretradecane (Cyclam S),

Sodium 2,3 dimercaptopropane-1-sulfonate (DMPS),

N-acetylpenicillamine (NAPA),

D-Penicillamine (PA),'

Desferroxamine,

2,3-dimercaptopropanol (BAL),

2,3-dimercaptosuccinic acid (DMSA),

trithiomolybdate,

3-7-Diazanonan-1,9-diamin (BE 6184),

1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid,

1,4,8,11-tetraazabicyclo[6.6.2]hexadecane,

4,11-bis(N,N-diethyl-amidomethyl)-1,4,8.11-tetraazabicyclo[6.6.2]hexadecane,

4,11-bis(amidoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane

melatonin,

clioquinol,

cuprizone,

N,N'-diethyldithiocarbamate,

zinc acetate,

zinc salts,

bathocuproinedisulfonic acid; bathocuprinedisulfonate,

neocuproine (2,9-dimethyl-1, 10-phenanthroline),

tetrathiomolybdate,

trimetazidine,

triethylene tetramine tetrahydrochloride,

2,3,2-tetraamine,

pyridine-2,6-bis(thiocarboxylic acid) or pyrrolidine dithiocarbamate,

tetraethylenepentamine,

N,N,N',N-tetrakis(2-pyridylemethyl) ethylenediamine

1,4,7,11-tetraazaundecane tetrahydrochloride,
tetraethylenepentamine pentahydrochloride,
D-Penicillamine (DPA),
1,10-orthophenanthroline,
3,4-Dihydroxybenzoic acid,
2,2'-bicinchinonic acid,
diamsar,
3, 4', 5, trihydroxystilbene (resveratrol),
mercaptodextran,
o-phenanthroline,
disulfiram (antabuse),
sar,
calcium trisodium diethylenetriaminepentaacetate (salt of cpd above), and
methimazole (1-methyl-2-thiolimidazole).

Preferably the one or more agent capable of decreasing the iron values content of the patient, if a chelator, preferably has a preferential affinity for iron values over the values of other trace metals (such as zinc and/or manganese). It is particularly important not to induce diseases of such transition metal deficiencies, eg; anaemia.

Administration of any iron chelator can be by a variety of routes including parenteral and oral. With such agents a dose rate for oral administration is about 10 times that for parenteral administration because of the lower bioavailablity of the drug. With, for example, trientine expressed as the dihydrochloride salt a suitable parenteral or oral dose is about 4g/day or below. A preferred dosage is from 1mg to 4g per day. Most preferably about 1.2g/day is delivered from a suitable dosage form.

Accordingly where the chelator is trientine as the dihydrochloride salt (and irrespective of excipients, diluents, carriers and vehicles) the dosage or dosages in a human patient, if parenteral, is to provide about 1.2mg/day, and if oral, about 1.2mg/day (i.e. the oral dosage may be ten times that of the parenteral dose provided to deliver 1.2mg/day).

Alternatively (and/or additionally) the agent capable of reducing iron values is a zinc salt (preferably as a flavoured aqueous solution) or trithiomolybdate (also a chelator). Suitable zinc salts include

- zinc acetate
- zinc chloride
- zinc sulphate
- zinc salts of intermediates of the citric acid cycle; such as citrate, isocitrate, ketoglutarate, succinate, malate
- zinc gluconate

With the preferred chelators herein referred to and the suitable salts of zinc it is possible to selectively decrease the iron values in the body as a whole (we believe without reaching depletion states for other transition metals) even though we believe without wishing to be bound there is little decrease in iron values in the intra cellular tissue, ie; the decrease is primarily extra cellular (eg; interstites, on the exterior of cells and/or on collagen).

In a first aspect the present invention is a method of improving tissue repair in a mammalian patient of damaged tissue selected from that of the myocardium, the vascular tree and organs dependent on the vascular tree, said method comprising or including the step of subjected the patient to, and/or administering to the patient, an agent or agents effective in lowering the iron values content of the patient's body sufficient to improve tissue repair.

Preferably the patient is not suffering from Wilson's Disease yet may have an elevated copper values content.

Preferably there is at least one iron values status determination.

In one preferment the agent is an iron chelation agent.

The patient can be a human being suffering from Type II diabetes Mellitus.

We believe the improvement of the tissue repair arises from a restoration of, or substantial restoration, of normal tissue stem cell responses.

The agent(s) may be selected from

trientine (triene), [more a copper chelator],

ethylenediaminetetraacetic acid (EDTA),
diethylenetriaminetetraacetic acid (DPTA),
2,2,2 tetramine tetrahydrochloride (TETA),
2,3,2 tetramine tetrahydrochloride,
D-penicillamine (DPA)
1,4,8,11 tetraazacyclotretradecane (Cyclam),
5,7,7',12,14,14' hexamethyl-1,4,8,11 tetraazacyclotretradecane (Cyclam S),
Sodium 2,3 dimercaptopropane-1-sulfonate (DMPS),
N-acetylpenicillamine (NAPA),
D-Penicillamine (PA),'
Desferroxamine,
2,3-dimercaptopropanol (BAL),
2,3-dimercaptosuccinic acid (DMSA),
trithiomolybdate,
3-7-Diazanonan-1,9-diamin (BE 6184),
1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid,
1,4,8,11-tetraazabicyclo[6.6.2]hexadecane,
4,11-bis(N,N-diethyl-amidomethyl)-1,4,8.11-tetraazabicyclo[6.6.2]hexadecane,
4,11-bis(amidoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane
melatonin,
clioquinol,
cuprizone,
N,N'-diethyldithiocarbamate,
zinc acetate,
zinc salts,
bathocuproinedisulfonic acid; bathocuprinedisulfonate,
neocuproine (2,9-dimethyl-1, 10-phenanthroline),
tetrathiomolybdate,
trimetazidine,
triethylene tetramine tetrahydrochloride,

2,3,2-tetraamine,
pyridine-2,6-bis(thiocarboxylic acid) or pyrrolidine dithiocarbamate,
tetraethylenepentamine,
N,N,N',N-tetrakis(2-pyridylemethyl) ethylenediamine
1,4,7,11-tetraazaundecane tetrahydrochloride,
tetraethylenepentamine pentahydrochloride,
D-Penicillamine (DPA),
1,10-orthophenanthroline,
3,4-Dihydroxybenzoic acid,
2,2'-bicinchinonic acid,
diamsar,
3, 4', 5, trihydroxystilbene (resveratrol),
mercaptodextran,
o-phenanthroline,
disulfiram (antabuse),
sar,
calcium trisodium diethylenetriaminepentaacetate (salt of cpd above), and
methimazole (1-methyl-2-thiolimidazole).

The agent (agents) can be a zinc salt (zinc salts).

The damage preferably is that that has arisen from any one or more of:

(i) disorders of the heart muscle (cardiomyopathy or myocarditis) such as idiopathic cardiomyopathy, metabolic cardiomyopathy which includes diabetic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy,

or

(ii) atheromatous disorders of the major blood vessels (macrovascular disease)
such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries,

or

(iii) toxic, drug-induced, and metabolic (including hypertensive and/or diabetic disorders of small blood vessels (microvascular disease) such as the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems,

or

(iv) plaque rupture of atheromatous lesions of major blood vessels such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the fermoral arteries and the popliteal arteries.

The patient may be suffering from and/or be predisposed to heart failure.

The patient may be (and preferably is) suffering from Type II diabetes Mellitus.

In another aspect the invention is **the use of a compound (a) which itself *in vivo* or (b) which has at least one metabolite *in vivo* which is (i) an iron chelator or (ii) otherwise reduces available iron values** for the production of a pharmaceutical composition or dosage unit able to reduce the level of iron in a mammal thereby to elicit by a lowering of iron values in a mammalian patient an improvement of tissue repair of damaged tissue selected from that of the myocardium, the vascular tree and organs dependent on the vascular tree.

The damage may be that which has arisen from a disease selected from the group:

(i) **disorders of the heart muscle** (cardiomyopathy or myocarditis) such as idiopathic cardiomyopathy, metabolic cardiomyopathy which includes diabetic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy,

or

(ii) atheromatous disorders of the major blood vessels (**macrovascular disease**)

such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries,

or

(iii) toxic, drug-induced, and metabolic (including hypertensive and/or diabetic disorders of small blood vessels (**microvascular disease**) such as the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems,

or

(iv) **plaque rupture of atheromatous lesions of major blood vessels** such as

the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the fermoral arteries and the popliteal arteries.

The compound is preferably selected from

trientine (triene), [more a copper chelator]
ethylenediaminetetraacetic acid (EDTA),
diethylenetriaminetetraacetic acid (DPTA),
2,2,2 tetramine tetrahydrochloride (TETA),
2,3,2 tetramine tetrahydrochloride,
D-penicillamine (DPA)
1,4,8,11 tetraazacyclotretradecane (Cyclam),
5,7,7',12,14,14' hexamethyl-1,4,8,11 tetraazacyclotretradecane (Cyclam S),
Sodium 2,3 dimercaptopropane-1-sulfonate (DMPS),
N-acetylpenicillamine (NAPA),
D-Penicillamine (PA),'
Desferroxamine,
2,3-dimercaptopropanol (BAL),

2,3-dimercaptosuccinic acid (DMSA),
trithiomolybdate,
3-7-Diazanonan-1,9-diamin (BE 6184),
1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid,
1,4,8,11-tetraazabicyclo[6.6.2]hexadecane,
4,11-bis(N,N-diethyl-amidomethyl)-1,4,8.11-tetraazabicyclo[6.6.2]hexadecane,
4,11-bis(amidoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane
melatonin,
clioquinol,
cuprizone,
N,N'-diethyldithiocarbamate,
zinc acetate,
zinc salts,
bathocuproinedisulfonic acid; bathocuprinedisulfonate,
neocuproine (2,9-dimethyl-1, 10-phenanthroline),
tetrathiomolybdate,
trimetazidine,
triethylene tetramine tetrahydrochloride,
2,3,2-tetraamine,
pyridine-2,6-bis(thiocarboxylic acid) or pyrrolidine dithiocarbamate,
tetraethylenepentamine,
N,N,N',N-tetrakis(2-pyridylemethyl) ethylenediamine
1,4,7,11-tetraazaundecane tetrahydrochloride,
tetraethylenepentamine pentahydrochloride,
D-Penicillamine (DPA),
1,10-orthophenanthroline,
3,4-Dihydroxybenzoic acid,
2,2'-bicinchinonic acid,
diamsar,
3, 4', 5, trihydroxystilbene (resveratrol),

mercaptodextran,

o-phenanthroline,

disulfiram (antabuse),

sar,

calcium trisodium diethylenetriaminepentaacetate (salt of cpd above), and methimazole (1-methyl-2-thiolimidazole).

Preferably the compound is trientine or a trientine type copper chelation agent.

Preferably the use involves pharmaceutically acceptable excipients, diluents and/or carriers.

The invention is also a dosage unit resulting from the use.

In another aspect the invention is **a method of treating a mammalian patient (eg; a human being) at risk of developing, with suspected or with actual tissue damage to the myocardium, the vascular tree and/or organs dependent on the vascular tree**, which method comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of decreasing the copper values content of the patient thereby to better enable tissue repair.

In still another aspect the invention is **a method of treating a mammalian patient (eg; a human being) at risk of developing, with suspected or with actual tissue disease to the myocardium, the vascular tree and/or organs dependent on the vascular tree**, which method comprises or includes the steps of

determining the iron status of the patient, and

if the iron status of a patient is elevated or at least normal, subjecting the patient to and/or administering to the patient one or more agents capable of decreasing the patient's iron values content thereby to better enable tissue repair.

The method may involve continual monitoring of the iron status of the patient.

The determination of the iron status can be by reference to extra cellular iron values.

Preferably the decreasing of the patient's iron values content is from an elevated status being that typical of the iron values status of a human patient suffering from Type II diabetic Mellitus over that of a non sufferer.

The method may include a copper values determination.

The method may include the step of diagnosing and/or monitoring hypertension.

The method may include the step of diagnosing alcoholism.

The method may include the step of diagnosing and/or monitoring a glucose mechanism abnormality of the patient.

Preferably the abnormality is Type II Diabetes mellitus, IGT and/or IFG.

The method can include the step of diagnosing and/or monitoring macrovascular, microvascular, toxic and/or metabolic damage in the patient.

The damage can be that of any one or more of:

(i) **disorders of the heart muscle** (cardiomyopathy or myocarditis) such as idiopathic cardiomyopathy, metabolic cardiomyopathy which includes diabetic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy,

or

(v) atheromatous disorders of the major blood vessels (**macrovascular disease**)

such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries,

or

(vi) toxic, drug-induced, and metabolic (including hypertensive and/or diabetic disorders of small blood vessels (**microvascular disease**) such as the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems,

or

(vii) **plaque rupture of atheromatous lesions of major blood vessels** such as

the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the fermoral arteries and the popliteal arteries.

In another aspect the present invention consists in **a method of treating a mammalian patient (eg; a human being) at risk of developing, with suspected or with actual disease to the myocardium, the vascular tree and/or organs dependent therefrom**, which method comprises or includes the step of subjecting the patient to and/or administering to the patient one or more agents capable of decreasing the iron values content of the patient.

In another aspect the present invention consists in **a method of treating a mammalian patient (eg; a human being) at risk of developing, with suspected or with actual disease to the myocardium, the vascular tree and/or organs dependent on the vascular tree**, which method comprises or includes the steps of

(i) determining the iron status of the patient, and

(ii) if the iron status of a patient is above that of a normal patient or is at least that of a normal patient, subjecting the

patient to and/or administering to the patient one or more agents capable of decreasing the iron values content.

Preferably the method involves continual monitoring of the iron status of the patient.

Preferably the determination of the iron status is by reference to extra cellular iron values.

Preferably the subjection or administration is with any one or more of the agents as hereinafter defined, preferred and/or exemplified.

In another aspect the present invention is **the use of a compound (a) which itself *in vivo* or (b) which has at least one metabolite *in vivo* which is a iron chelator or otherwise reduces available iron values** for the production of a pharmaceutical composition able to reduce the level of iron in a mammal (eg; in heart

tissue and/or in the walls of major blood vessels respectively) for the treatment (e.g. by repair of tissue resulting) of a disease of any one or more of the kinds defined herein.

In another aspect the invention is **a method of improving tissue repair in a mammalian patient not suffering from Wilson's Disease yet having an elevated copper and iron values body content**, said method comprising or including the step of subjected the patient to, and/or administering to the patient, an agent effective in lowering the copper and iron values content of the patient's body sufficient to improve tissue repair (eg. by restoration or substantially restoration of normal tissue stem cell responses).

Preferably there is at least one iron and/or copper values status determination.

In another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual diabetic cardiomyopathy** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of decreasing the iron values content of the patient.

Such agent(s) may comprise or include copper and iron chelators and/or may include compounds or compositions otherwise capable of decreasing the copper and iron values content of the patient (for example; zinc (eg; as a suitable salt such as the gluconate).

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method include diagnosis of the patient as a diabetic.

In another aspect the present invention consists in a **method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual diabetic acute myocardial infarction** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of decreasing the iron values content of the patient.

Such agent(s) may comprise or include iron chelators and/or may include compounds or compositions otherwise capable of decreasing the iron values content of the patient (for example; zinc (eg; as a suitable salt such as the gluconate).

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method includes diagnosis of the patient as a diabetic.

In still another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual diabetic hypertensive cardiomyopathy** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient one or more agents capable of decreasing the iron values content of the patient.

In yet another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual acute myocardial infarction (AMI) associated with impaired glucose tolerance (IGT)** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient one or more agents capable of reducing the iron values content of the patient.

The method includes one or both of the additional steps of diagnosis of the patient with myocardial infarction and/or impaired glucose tolerance.

In another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual acute myocardial infarction associated with impaired fasting glucose (IFG)** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of reducing the iron values content of the patient.

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method include diagnosis of the patient as a diabetic.

The method includes one or both of the additional steps of diagnosis of the patient with myocardial infarction and/or impaired fasting glucose.

In still another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual hypertensive cardiomyopathy associated with IGT** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of reducing the iron values content of the patient.

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method include diagnosis of the patient as a diabetic.

The method preferably includes the additional steps of diagnosing the patient as a hypertensive and/or as being subjected to IGT and/or suffering from actual hypertensive cardiomyopathy.

In yet another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual hypertensive cardiomyopathy associated with IFG** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of reducing the iron values content of the patient.

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method include diagnosis of the patient as a diabetic.

The method may include the additional step or steps of diagnosing the patient as a hypertensive and/or having IFG and/or having hypertensive cardiomyopathy.

In another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual ischaemic cardiomyopathy associated with IGT** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of reducing the copper values content of the patient.

Such agent(s) may comprise or include copper chelators and/or may include compounds or compositions otherwise capable of decreasing the copper values content of the patient (for example; zinc (eg; as a suitable salt such as the gluconate) or tri thiomolybdate (also a copper chelator) which tend to prevent copper absorption by a patient).

The method may include an additional step or steps of monitoring the copper values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method includes diagnosis of the patient as a diabetic.

The method may include the additional step of determining the patient is subject to ischaemic disease and/or is subject to IGT and/or is suffering from ischaemic cardiomyopathy.

In yet another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual ischaemic cardiomyopathy associated with IFG** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of decreasing the iron values content of the patient.

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method include diagnosis of the patient as a diabetic.

The method may include the additional step or steps of diagnosing the patient as ischaemic and/or having IFG and/or suffering from ischaemic cardiomyopathy.

The method may include the additional step or steps of diagnosing the patient as subject to ischaemic disease and/or suffering from coronary heart disease (CHD) and/or suffering from ischaemic cardiomyopathy.

In another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual ischaemic cardiomyopathy associated with coronary heart disease (CHD)** which comprises or includes the step of subjecting the patient mammal to and/or

administering to the patient mammal one or more agents capable of decreasing the iron values content of the patient.

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method include diagnosis of the patient as a diabetic.

The method may include the additional step or steps of diagnosing the patient as suffering from acute myocardial infarction.

In another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual acute myocardial infarction not associated with any abnormality of the glucose metabolism** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of decreasing the iron values content of the patient.

The method may include an additional step or steps of monitoring the copper values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

The method may include the additional step or steps of diagnosing the patient as hypertensive and/or suffering from hypertensive cardiomyopathy.

In another aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual hypertensive cardiomyopathy not associated with any abnormality of the glucose metabolism** which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient one or more agents capable of decreasing the iron values content of the patient.

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method includes diagnosis of the patient as a diabetic.

The method may include the additional step or steps of diagnosing the patient as hypertensive and/or suffering from hypertensive cardiomyopathy.

In yet a further aspect the present invention consists in **a method of treating a mammal (eg; a human being) at risk of developing, with suspected or with actual ischaemic cardiomyopathy not associated with any abnormality of the glucose metabolism** (irrespective of whether or not such ischaemic cardiomyopathy is associated with coronary heart disease or not) which comprises or includes the step of subjecting the patient mammal to and/or administering to the patient one or more agents capable of decreasing the iron values content of the patient.

The method may include an additional step or steps of monitoring the iron values of the patient prior to, simultaneously with and/or subsequent to the patient being subjected to or being administered with the agent(s).

Preferably said method include diagnosis of the patient as a diabetic.

The method may include the additional step or steps of diagnosing the patient as suffering from ischaemic disease and/or ischaemic cardiomyopathy.

In a further aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of:

(i) **categorising** the human by reference to

(a) whether suffering from one or more of Type II diabetes, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), and/or

(b) iron status, and

(ii) (provided the patient (a) is suffering from Type II diabetes and/or (IGT) and/or IFG, and/or (b) is not biochemically or clinically iron deficient) **subjecting** the patient to a regimen with a view to decreasing the presence of iron values.

Preferably there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron decreasing regimen.

In a further aspect the present invention consists in **a method of treating a human at risk to developing, with suspected or with actual acute myocardial infarction** which comprises or includes the steps of:

i) **categorising** the human by reference to

(a) whether suffering from one or more Type II diabetes, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), and/or

(b) iron status, and

ii) (provided the patient (a) is suffering from Type II diabetes and/or IGT and/or IGF, and/or (b) is not biochemically or clinically iron deficient) **subjecting** the patient to an iron chelation and/or other iron values decreasing regimen with a view to decreasing the presence of iron.

Preferably step (i) also includes reference to (c) heart function. Alternatively and/or additionally benefit to patient is assessed by reference to heart function.

In another aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual hypertensive cardiomyopathy** which comprises or includes the steps of:

(i) **categorising** the human by reference to

(a) whether hypertensive, and/or (b) iron status; and

(ii) **subjecting** the patient to an iron chelation and/or other iron values decreasing regimen with a view to decreasing the presence of iron whilst preferably ensuring patient does not have or does not develop an iron deficiency.

Preferably step (i) also includes one or both references to (b) iron status and/or (c) heart function.

Preferably there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron chelation regimen.

In a further aspect the present invention consists in **a method of treating a human at risk to developing, with suspected or with actual ischaemic cardiomyopathy** which comprises or includes the steps of:

(i) **categorising** the human by reference to

(a) whether suffering from ischaemia, and/or (b) iron status; and

(ii) **subjecting** the patient to an iron chelation and/or other iron values decreasing regimen with a view to decreasing the presence of iron whilst preferably ensuring patient does not have or does not develop any iron deficiency.

In still another aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of:

(i) **categorising** the human as a candidate patient by reference to at least

(a) whether suffering from Type II diabetes, (IGT), impaired fasting glucose (IFG) and/or hypertensive impaired glucose tolerance, and

(b) heart function, and

(ii) **subjecting** the patient to an iron chelation and/or other iron values decreasing regimen with a view to decreasing the presence of copper whilst preferably ensuring patient does not have or does not develop any iron deficiency.

Preferably there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron chelation regimen.

In a further aspect the present invention consists in **a method of treating a human or other mammal at risk to developing, with suspected or with actual (I) arterial, (II) arterial and coronary and/or other organ, and/or (III) heart muscle disease** which comprises or includes the steps of:

(i) **categorising** the human or other mammal as a candidate patient and

(ii) **subjecting** the patient to an iron chelation and/or other iron values decreasing regimen with a view to decreasing the presence of iron.

Preferably step (i) includes a determination of the iron status of the human or other mammal.

Preferably there is a step (iii) of ensuring by reference to heart and/or arterial function that the patient is benefiting from the iron chelation and/or other iron values decreasing regimen.

In another aspect the present invention consists in **a method of treatment reliant upon the methodology of either Figures 3 or 4 of the accompanying drawings.**

In a further aspect the present invention consists in **a method of treating a human having Type II diabetes or impaired glucose intolerance at risk of developing, with suspected or with actual cardiomyopathy** which comprises or

includes subjecting the patient to an iron chelation regimen with a view to decreasing the presence of chelatable iron to heart tissue whilst at least on occasions having monitored and/or monitoring the patient to avoid an iron deficit.

Preferably said patient has been categorised to ensure that the regime is not commenced and/or does not continue should the patient be iron deficient and/or suffering from iron deficiency anaemia.

Preferably the patient is categorised to exclude classical iron overload, ie; the patient to be subjected to the iron chelation regime is one with chelatable iron in heart tissue that may be present above normal levels of such chelatable iron in heart tissues and not one subject to chelation or other therapy to reduce total iron level.

In a further aspect the present invention consists in **a method of treating a human having Type II diabetes or impaired glucose intolerance at risk of developing, with suspected or with actual macrovascular disease** which comprises or includes subjecting the patient to a total body iron content decreasing regimen.

Preferably said patient has been categorised to ensure that the regimen is not commenced and/or does not continue should the patient be iron deficient and/or suffering from iron deficiency anaemia.

In still a further aspect or as one preferment the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy related heart failure** which comprises or includes decreasing the levels of chelatable and/or other iron values of such patient preferably without taking the patient into iron deficit.

In yet a further aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual macrovascular disease** of the arterial tree which comprises or includes decreasing the levels of chelatable iron in the walls of major blood vessels of such patient without taking the patient into iron deficit.

In yet another aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of

(i) categorising the human as being at risk of developing, with suspected or with actual cardiomyopathy, and

(ii) subjecting the patient to an iron chelation regimen with a view to decreasing the presence of iron.

Preferably said iron chelation regimen is subject to monitoring to ensure the patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

Preferably said categorisation relies on an initial check of heart function and the patient being categorised for the iron chelation regimen when that heart function is below normal.

Preferably the heart function monitoring continues into or beyond the iron chelation regimen.

Preferably said categorisation includes a determination of the patient suffering from Type II diabetes or impaired glucose tolerance.

Preferably said categorisation involves a reference to iron status of the patient prior to any commencement or substantial duration of the iron chelation regimen to ensure the patient does no have or does not develop an iron deficiency and/or iron deficiency anaemia.

In any of the foregoing procedures the following preferments (any one, some or all) arise:

Preferably said compound is an iron chelator which in the mammal is substantially without an ability to generate free radicals in significant qualities and which also in the mammal at the dosage regimen to be given will not chelate iron (and preferably copper) down to a depletion state in the mammal.

Preferably the regimen is in concert (serial, simultaneous or otherwise) with a regimen to antagonise fructosamine oxidase.

Preferably the dosage unit(s) is (are) the dosage unit(s) of a copper and/or iron decreasing regimen.

The regimen may run in concert with any of the regimens disclosed in WO 00/18392.

Preferably said use involves pharmaceutically acceptable diluents and/or carriers.

Preferably the composition is for use in a method as previously defined.

The present invention also consists in **a dosage unit** resulting from any such use.

In another aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of:

(i) **categorising** the human by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(b) iron status, and

(ii) (provided the patient (a) is suffering from Type II diabetes, and (b) is not biochemically or clinically iron deficient, is not suffering from iron deficiency anaemia, and is not subject to classical iron overload) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in heart tissue.

Preferably such decrease reduces iron available in such tissue available to catalyse a Haber-Weiss Reaction.

Preferably there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron chelation regime.

In a further aspect the present invention consists in **a method of treating a human at risk to developing, with suspected or with actual macrovascular disease** which comprises or includes the steps of:

i) **categorising** the human by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(b) iron status, and

ii) (provided the patient (a) is suffering from Type II diabetes and (b) is not biochemically or clinically iron deficient, is not suffering from iron deficiency

anaemia, and is not subject to classical iron overload) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the walls of major blood vessels (eg; as might be available to catalyse a Haber-Weiss Reaction).

Preferably step (i) also includes reference to (c) heart function. Alternatively benefit to patient is assessed by reference to heart function.

In another aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of:

(i) **categorising** the human by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(ii) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in heart tissue whilst ensuring patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

Preferably step (i) also includes one or both references to (b) iron status and/or (c) heart function.

Preferably there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron chelation regime.

In a further aspect the present invention consists in **a method of treating a human at risk to developing, with suspected or with actual macrovascular disease** which comprises or includes the steps of:

(i) **categorising** the human by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(ii) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the walls of major blood vessels whilst ensuring patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

In still another aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of:

(i) **categorising** the human as a candidate patient by reference to at least

(a) whether suffering from Type I or II diabetes or impaired glucose tolerance, and

(b) heart function, and

(ii) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in heart tissue whilst ensuring patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

Preferably there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron chelation regime.

In a further aspect the present invention consists in **a method of treating a human at risk to developing, with suspected or with actual macrovascular disease** which comprises or includes the steps of:

(i) **categorising** the human as a candidate patient by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(b) iron status, and/or

(c) heart function

(ii) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the walls of major blood vessels whilst ensuring (eg; by at least some testing) patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

Preferably there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron chelation regime.

In a further aspect the present invention consists in **a method of treating a human having Type II diabetes or impaired glucose intolerance at risk of developing, with suspected or with actual macrovascular disease** which comprises

or includes subjecting the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the walls of major blood vessels whilst at least on occasions having monitored and/or monitoring the patient to avoid an iron deficit.

Preferably said patient has been categorised to ensure that the regime is not commenced and/or does not continue should the patient be iron deficient and/or suffering from iron deficiency anaemia.

Preferably the patient is categorised to exclude classical iron overload, ie; the patient to be subjected to the iron chelation regime is one with chelatable iron in the walls of major blood vessels that may be present above normal levels of such chelatable iron in the walls of major blood vessels and not one subject to chelation or other therapy to reduce to iron level.

In still a further aspect or as one preferment the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy related heart failure** which comprises or includes reducing the levels of chelatable iron in the heart tissue of such patient without taking the patient into iron deficit.

In yet a further aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual macrovascular disease** which comprises or includes reducing the levels of chelatable iron in the walls of major blood vessels of such patient without taking the patient into iron deficit.

In yet another aspect the present invention consists in **a method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of

(i) categorising the human as being at risk of developing, with suspected or with actual cardiomyopathy, and

(ii) subjecting the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the patient's heart tissue.

Preferably said iron chelation regime is subject to monitoring to ensure the patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

Preferably said categorisation relies on an initial check of heart function and the patient being categorised for the iron chelation regime when that heart function is below normal.

Preferably the heart function monitoring continues into or beyond the iron chelation regime.

Preferably said categorisation includes a determination of the patient suffering from Type II diabetes or impaired glucose tolerance.

Preferably said categorisation involves a reference to iron status of the patient prior to any commencement or substantial duration of the iron chelation regime to ensure the patient does no have or does not develop an iron deficiency and/or iron deficiency anaemia.

In any of the foregoing procedures the following preferments (any one, some or all) arise:

- said compound is an iron chelator which in the mammal is substantially without an ability to generate free radicals in significant qualities and which also in the mammal at the dosage regime to be given will not chelate iron down to a depletion state in the mammal,
- the subjection is at a dosage regime less than that which, for a patient suffering from classical iron overload, would have the effect of reducing the iron levels of that patient to normal,
- the subjection is also at a dosage regime (ie; whether dependent upon dosage unit(s) and/or frequency) which does not or will not reduce a patient of normal iron levels to a deficiency state and/or to anaemia,
- the regime is in concert (serial, simultaneous or otherwise) with a regime to antagonise fructosamine oxidase,
- the regime to antagonise fructosamine oxidase relies upon copper chelation,
- the dosage unit(s) is (are) the dosage unit(s) of the iron chelation regime also (e.g. any of the other regimes disclosed in WO 00/18392).

In another aspect the present invention is **the use of a compound (a) which itself *in vivo* or (b) which has at least one metabolite *in vivo* which is an iron chelator** for the production of a pharmaceutical composition able to reduce the level of iron in heart tissue and/or in the walls of major blood vessels respectively for the treatment of diabetic cardiomyopathy and/or diabetic macrovascular disease.

Preferably the composition is for use in a method as previously defined.

The iron chelator (in some cases also a copper chelator) is preferably selected from the following preferments.

Preferably the chelation is with one or more of the preferred compounds hereafter referred to:

Preferably the compound is trientine.

Preferably the compound is a derivative of trientine.

Preferably the compound is a salt of trientine.

Preferably the compound is a hydrochloride salt of trientine.

Preferably the compound is the dihydrochloride salt of trientine.

Preferably the compound is desferrioxamine.

Preferably the compound is deferoxamine mesylate.

Preferably the compound is mimosine.

Preferably the compound is bathocuproine sulphonate.

Preferably the compound is bathophenanthroline sulphonate.

Preferably the compound is 1,2-diethyl-3-hydroxypyridine-4-one.

Preferably the compound is O-Trensox.

Preferably the compound is N,N'-Bis(3,4,5-trimethoxybenzyl)ethylenediamine-N,N'-diacetic acid.

Preferably the compound is N,N'-Bis(3,4,5-trimethoxybenzyl)ethylenediamine-N,N'-diacetic acid diacetoxymethyl ester.

Preferably the compound is desferri-exochelin 772SM.

Preferably the compound is deferiprone (1,2-dimethyl-3-hydroxypyridine-4-one).

Preferably the compound is diethylenetriaminepentaacetic acid (DETAPAC).

Preferably the compound is hydroxyethyl starch-deferoxamine mesylate (HES-DFO).

Preferably the compound is desferrithiocin.

Preferably the compound is 4, 5-dihydro-2-(2-hydroxyphenyl)-4-thiazolecarboxylic acid (desmethyldesferrithiocin, DMDFT).

Preferably the compound is 4,5-dihydro-2-(2,4-dihydroxyphenyl)-4-thiazolecarboxylic acid [4-(s)-hydroxydesaza-DMDFT.

Preferably the compound is 2-(2-hydroxyphenyl)-4-oxazolinecarboxylic acid.

Preferably the compound is 2-pyridylcarboxaldehyde isonicotinoyl hydrazone.

Preferably the compound is 2-pyridylcarboxaldehyde benzoyl hydrazone (PCBH).

Preferably the compound is 2-pyridylcarboxaldehyde m-bromobenzoyl hydrazone (PCBBH).

Preferably the compound is 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone (PCTH).

Preferably the compound is isonicotinoyl salicylaldehyde hydrazone.

Preferably the compound is 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone (NIH).

Preferably the compound is ((+)-3-hydroxy-1-(2-hydroxyethyl)-2-hydroxyphenyl-methyl-1H-pyridin-4-one).

Preferably the compound is dexrazoxane (razoxane; 4,4-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione.

Preferably the compound is CGP 75254A.

Preferably the compound is N,N'-bis(o-hydroxybenzyl) ethylenediamine-N,N'-diacetic acid (HBED).

Preferably the compound is N,N'-ethylenebis(o-hydroxyphenylglycine) (EHPG).

Preferably the compound is 2,3-dihydroxybenzoic acid (2,3-DHB).

This invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more of said parts, elements or

features, and where specific integers are mentioned herein which have known equivalents in the art to which this invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

DESCRIPTION OF THE DRAWINGS

Preferred forms of the present invention will now be described with reference to any one or more of the following drawings in which,

Figure 1 is a diagram showing what we believe to be a pathway to cardiomyopathy,

Figure 2 is our hypothesis of the mechanisms involved applicable to cardiomyopathy and macrovascular disease in a patient with Type II diabetes or impaired glucose tolerance, such a hypothesis showing reliance on a possible fructosamine oxidase/superoxide dismutase generation of a precursor to an iron catalysed reaction (the Haber-Weiss Reaction) which generates the harmful free radicals,

Figure 3 is a methodology for a human patient with suspected cardiomyopathy reliant upon at least step 3 of the hypothesis of Figure 2,

Figure 4 is a similar diagram to that of Figure 3 but this time in respect of a patient with suspected macrovascular disease,

Figure 5 shows a significant increase in iron concentrations in the heart of untreated diabetic animals compared with non-diabetic animals, and that tissue iron levels are decreased after treatment with triene,

Figure 6 shows a significant increase in urinary iron excretion in untreated diabetic animals compared with non-diabetic animals, and that urinary iron excretion is markedly increased following treatment with triene,

Figure 7 shows a grading of cardiomyopathy in untreated diabetic animals compared with non-diabetic animals, wherein cardiomyopathic changes are markedly decreased following treatment with triene,

Figure 8 shows the appearance of the myocardium corresponding with the severity grades shown in Figure 7,

Figure 9 shows Masson Trichrome Staining of Heart Tissues for a non diabetic *Wistar* rate, a Triene treated diabetic *Wistar* rat, and an untreated diabetic *Wistar* rat,

Figure 10 shows average light density with respect to Figure 9 for each of the categories non diabetic, diabetic and triene treated diabetic.

Example 1:

1. **Procedure**

Diabetes was induced in 32 of 40 male Wistar rats aged 6-8 weeks & weighing 200-300g by administering Streptozocin (STZ) 60mg/kg body weight in citrate buffer pH 4.5 by intraperitoneal injection. Control rats were administered a sham injection of citrate buffer. Diabetes was confirmed by fasting glucose >15mmol/L seven days after STZ treatment. Animals were randomized into 2 control & 1 treatment group: (i) Non-diabetic control (8 rats); (ii) Diabetic controls (24 rats); & (iii) Treated with triene (8 rats). Animals were fed standard rat chow *ad libitum*. Diabetic rats were treated by adding triene, 50mg/L to drinking water. Weight was monitored and diabetic rats were treated with alternate daily subcutaneous injections of ultralente insulin (4U) to maintain body growth and enhance survival. Plasma glucose levels were measured on tail-vein blood samples obtained at monthly intervals. Glycated haemoglobin was measured by HPLC affinity chromatography. Creatinine and iron were measured on overnight urine collected at monthly intervals. At the termination of the study, rats were anaesthetised with nitrous oxide and heart and other & tissues were harvested, fixed in formalin for light microscopy, and then processed in paraffin. Two-micrometer sections were cut and stained with haematoxylin and eosin and Massons Trichrome.

2. **Histology findings:**

Tissue samples were fixed in 4% Formaldehyde in phosphate buffer. The tissue was dehydrated, paraffin infiltrated and embedded in wax. Ultra thin sections were cut using a Microm HM330 Rotary microtome. Heart sections were cut longitudinally with the heart wall so that all sections of the myocardial walls including the

endocardium and epicardium sections, could be seen visualised. The following staining methods were used:

- Erlich's Haematoxylin and Eosin stain: Nuclei stained blue, cytoplasm - shades of pink.
- Elastic van Giesen stain: Elastic fibres - black, nuclei - blue, collagen - pink, muscle - brown.
- Masson trichrome stain: Nuclei - black, myofibrillar proteins - red, collagen/connective tissue and fibril free cytoplasm - green.

Histology results reveal a significant loss/drop in density of myofibers in sections of diabetic untreated hearts when compared to control sections. Triene treatment of diabetic rats appears to prevent this loss of myofibrillar proteins (See Figures 7, 8, 9, & 10). These results were confirmed by transmission electron microscopy.

3. **Tissue iron concentrations:** Small pieces of heart tissue (about 0.03g wet weight) were cut, washed in saline and placed in clay crucibles. Only tissue from the left and right ventricles was used, which meant each rat had two metal readings. The crucibles were baked in an oven for 20 hours at 110EC. The tissue was then weighed and placed in a 5ml test tube. 1 ml of 10M (69%) nitric acid was added to the tissue and was left overnight to digest. Once the tissue was fully digested 3 ml of MilliQ water was added. The samples were then analysed using a Perkin Elmer (PE) Model 31- Atomic Absorption Spectrophotometer in conjunction with a PE HGA-600 Graphtie Furnace and PE AS-60 Furnace Autosampler. Measurements were made with an Fe hollow-cathode lamp (Perkin Elmer Corporation) operated at 15 W using the 248.3 nm atomic line for iron and a slit width of 0.7 nm with deuterium background correction. Pyrolytically coated graphite tubes were used for all analyses. The injection volume was 20 uL. Results of iron analyses are shown in Figures 5 and 6.

WHAT WE CLAIM IS:

1. A method of improving tissue repair in a mammalian patient of damaged tissue selected from that of the myocardium, the vascular tree and organs dependent on the vascular tree, said method comprising or including the step of subjected the patient to, and/or administering to the patient, an agent or agents effective in lowering the iron values content of the patient's body sufficient to improve tissue repair.

2. A method of claim 1 wherein the patient has an elevated iron values content.

3. A method of claim 2 wherein there is at least one iron values status determination.

4. A method of claim 1 wherein the agent is an iron and/or an iron/copper chelation agent.

5. A method of claim 4 wherein trientine is administered at dosages or a dosage to provide, from 1mg to 4g in a human patient, when expressed as the dihydrochloride salt.

6. A method of claim 1 wherein the patient is a human being suffering from Type II diabetes Mellitus.

7. A method of claim 1 wherein improvement of the tissue repair arises from a restoration of, or substantial restoration, of normal tissue stem cell responses.

8. A method of claim 1 wherein the agent(s) is (are) selected from

trientine (triene),
ethylenediaminetetraacetic acid (EDTA),
diethylenetriaminetetraacetic acid (DPTA),
2,2,2 tetramine tetrahydrochloride (TETA),
2,3,2 tetramine tetrahydrochloride,
D-penicillamine (DPA)
1,4,8,11 tetraazacyclotretradecane (Cyclam),
5,7,7',12,14,14' hexamethyl-1,4,8,11 tetraazacyclotretradecane (Cyclam S),
Sodium 2,3 dimercaptopropane-1-sulfonate (DMPS),
N-acetylpenicillamine (NAPA),
D-Penicillamine (PA),'

Desferroxamine,
2,3-dimercaptopropanol (BAL),
2,3-dimercaptosuccinic acid (DMSA),
trithiomolybdate,
3-7-Diazanonan-1,9-diamin (BE 6184),
1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid,
1,4,8,11-tetraazabicyclo[6.6.2]hexadecane,
4,11-bis(N,N-diethyl-amidomethyl)-1,4,8.11-tetraazabicyclo[6.6.2]hexadecane,
4,11-bis(amidoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane
melatonin,
clioquinol,
cuprizone,
N,N'-diethyldithiocarbamate,
zinc acetate,
zinc salts,
bathocuproinedisulfonic acid; bathocuprinedisulfonate,
neocuproine (2,9-dimethyl-1, 10-phenanthroline),
tetrathiomolybdate,
trimetazidine,
triethylene tetramine tetrahydrochloride,
2,3,2-tetraamine,
pyridine-2,6-bis(thiocarboxylic acid) or pyrrolidine dithiocarbamate,
tetraethylenepentamine,
N,N,N',N-tetrakis(2-pyridylemethyl) ethylenediamine
1,4,7,11-tetraazaundecane tetrahydrochloride,
tetraethylenepentamine pentahydrochloride,
D-Penicillamine (DPA),
1,10-orthophenanthroline,
3,4-Dihydroxybenzoic acid,
2,2'-bicinchinonic acid,

diamsar,

3, 4', 5, trihydroxystilbene (resveratrol),

mercaptodextran,

o-phenanthroline,

disulfiram (antabuse),

sar,

calcium trisodium diethylenetriaminepentaacetate (salt of cpd above), and methimazole (1-methyl-2-thiolimidazole).

9. A method of claim 1 wherein the agent (agents) is (are) a zinc salt (zinc salts).

10. A method of claim 1 wherein the damage is that that has arisen from any one or more of:

(i) disorders of the heart muscle (cardiomyopathy or myocarditis) such as idiopathic cardiomyopathy, metabolic cardiomyopathy which includes diabetic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy,

or

(ii) atheromatous disorders of the major blood vessels (macrovascular disease)
such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries,

or

(iii) toxic, drug-induced, and metabolic (including hypertensive and/or diabetic disorders of small blood vessels (microvascular disease) such as the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems,

or

(iv) plaque rupture of atheromatous lesions of major blood vessels such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular

arteries, the renal arteries, the iliac arteries, the femoral arteries and the popliteal arteries.

11. A method of claim 1 wherein the patient is suffering from and/or is predisposed to heart failure.

12. A method of claim 11 wherein the patient is suffering from Type II diabetes Mellitus.

13. **The use of a compound (a) which itself *in vivo* or (b) which has at least one metabolite *in vivo* which is (i) an iron chelator or (ii) otherwise reduces available iron values** for the production of a pharmaceutical composition or dosage unit able to reduce the level of iron in a mammal thereby to elicit by a lowering of iron values in a mammalian patient an improvement of tissue repair of damaged tissue selected from that of the myocardium, the vascular tree and organs dependent on the vascular tree.

14. The use of claim 13 wherein the damage is that which has arisen from a disease selected from the group:

(i) **disorders of the heart muscle** (cardiomyopathy or myocarditis) such as idiopathic cardiomyopathy, metabolic cardiomyopathy which includes diabetic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy,

or

(ii) atheromatous disorders of the major blood vessels (**macrovascular disease**)

such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries,

or

(iii) toxic, drug-induced, and metabolic (including hypertensive and/or diabetic disorders of small blood vessels (**microvascular disease**) such as the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems,

or

(iv) **plaque rupture of atheromatous lesions of major blood vessels** such as

the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the fermoral arteries and the popliteal arteries.

15. The use of claim 13 or 14 wherein the compound is selected from

trientine (triene),

ethylenediaminetetraacetic acid (EDTA),

diethylenetriaminetetraacetic acid (DPTA),

2,2,2 tetramine tetrahydrochloride (TETA),

2,3,2 tetramine tetrahydrochloride,

D-penicillamine (DPA)

1,4,8,11 tetraazacyclotretradecane (Cyclam),

5,7,7',12,14,14' hexamethyl-1,4,8,11 tetraazacyclotretradecane (Cyclam S),

Sodium 2,3 dimercaptopropane-1-sulfonate (DMPS),

N-acetylpenicillamine (NAPA),

D-Penicillamine (PA),'

Desferroxamine,

2,3-dimercaptopropanol (BAL),

2,3-dimercaptosuccinic acid (DMSA),

trithiomolybdate,

3-7-Diazanonan-1,9-diamin (BE 6184),

1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid,

1,4,8,11-tetraazabicyclo[6.6.2]hexadecane,

4,11-bis(N,N-diethyl-amidomethyl)-1,4,8.11-tetraazabicyclo[6.6.2]hexadecane,

4,11-bis(amidoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane

melatonin,

clioquinol,

cuprizone,

N,N'-diethyldithiocarbamate,
zinc acetate,
zinc salts,
bathocuproinedisulfonic acid; bathocuprinedisulfonate,
neocuproine (2,9-dimethyl-1, 10-phenanthroline),
tetrathiomolybdate,
trimetazidine,
triethylene tetramine tetrahydrochloride,
2,3,2-tetraamine,
pyridine-2,6-bis(thiocarboxylic acid) or pyrrolidine dithiocarbamate,
tetraethylenepentamine,
N,N,N',N-tetrakis(2-pyridylemethyl) ethylenediamine
1,4,7,11-tetraazaundecane tetrahydrochloride,
tetraethylenepentamine pentahydrochloride,
D-Penicillamine (DPA),
1,10-orthophenanthroline,
3,4-Dihydroxybenzoic acid,
2,2'-bicinchinonic acid,
diamsar,
3, 4', 5, trihydroxystilbene (resveratrol),
mercaptodextran,
o-phenanthroline,
disulfiram (antabuse),
sar,
calcium trisodium diethylenetriaminepentaacetate (salt of cpd above), and methimazole (1-methyl-2-thiolimidazole).

16. The use of claim 13 wherein the compound is an iron and or an iron/copper chelation agent.

17. The use of claim 13 which involves pharmaceutically acceptable excipients, diluents and/or carriers.

18. A dosage unit resulting from the use of claim 13.

19. **A method of treating a mammalian patient (eg; a human being) at risk of developing, with suspected or with actual tissue disease to the myocardium, the vascular tree and/or organs dependent on the vascular tree**, which method comprises or includes the step of subjecting the patient mammal to and/or administering to the patient mammal one or more agents capable of decreasing the iron values content of the patient thereby to better enable tissue repair.

20. A method of claim 19 coupled with a determination that the patient's oron values status is elevated.

21. A method of claim 19 or 20 wherein the agent(s) is (are) a chelator (chelators) of iron.

22. A method of claim 21 wherein the agent(s) has (have) an affinity for copper over that of iron.

23. **A method of treating a mammalian patient (eg; a human being) at risk of developing, with suspected or with actual tissue disease to the myocardium, the vascular tree and/or organs dependent on the vascular tree**, which method comprises or includes the steps of

(i) determining the iron status of the patient, and

(ii) if the iron status of a patient is elevated or normal, subjecting the patient to and/or administering to the patient one or more agents capable of decreasing the patient's iron values content thereby to better enable tissue repair.

24. A method of claim 23 which involves continual monitoring of the iron status of the patient.

25. A method of claim 23 or 24 wherein the determination of the iron status is by reference to extra cellular iron values.

26. A method of claim 23 wherein the decreasing of the patient's iron values content is from an elevated status being that typical of the iron values status of a human patient suffering from Type II diabetes mellitus over that of a non sufferer.

27. A method of claim 23 which includes the step of diagnosing and/or monitoring hypertension.

28. A method of claim 23 which includes the step of diagnosing alcoholism.

29. A method of claim 23 which includes the step of diagnosing and/or monitoring a glucose mechanism abnormality of the patient.

30. A method of claim 29 wherein the abnormality is Type II Diabetes mellitus, IGT and/or IFG.

31. A method of claim 23 which includes the step of diagnosing and/or monitoring macrovascular, microvascular, toxic and/or metabolic damage in the patient.

32. A method of claim 23 wherein the damage is that of any one or more of:

(i) **disorders of the heart muscle** (cardiomyopathy or myocarditis) such as idiopathic cardiomyopathy, metabolic cardiomyopathy which includes diabetic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy,

or

(ii) atheromatous disorders of the major blood vessels (**macrovascular disease**)

such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the femoral arteries, and the popliteal arteries,

or

(iii) toxic, drug-induced, and metabolic (including hypertensive and/or diabetic disorders of small blood vessels (**microvascular disease**) such as the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems,

or

(iv) **plaque rupture of atheromatous lesions of major blood vessels** such as

the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the fermoral arteries and the popliteal arteries.

33. **A method of treating a human at risk of developing, with suspected or with**

actual cardiomyopathy which comprises or includes the steps of:

(i) **categorising** the human by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(b) iron status, and

(ii) (provided the patient (a) is suffering from Type II diabetes, and (b) is not biochemically or clinically iron deficient, is not suffering from iron deficiency anaemia, and is not subject to classical iron overload) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in heart tissue.

34. A method of claim 33 wherein there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron chelation regime.

35. **A method of treating a human at risk to developing, with suspected or with actual macrovascular disease** which comprises or includes the steps of:

i) **categorising** the human by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(b) iron status, and

ii) (provided the patient (a) is suffering from Type II diabetes and (b) is not biochemically or clinically iron deficient, is not suffering from iron deficiency anaemia, and is not subject to classical iron overload) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the walls of major blood vessels.

36. A method of claim 33, 34 or 35 which also includes in step (i) reference to (c) heart function.

37. **A method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of:

(i) **categorising** the human by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(ii) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in heart tissue whilst ensuring patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

38. A method of claim 37 wherein step (i) also includes one or both reference to (b) iron status and/or (c) heart function.

39. A method of claim 37 or 38 wherein there is a step (iii) of ensuring by reference to heart function that the patient is benefiting from the iron chelation regime.

40. A method of any one of the claims 37 to 39 wherein said chelation reduces iron available to a Haber-Weiss reaction.

41. **A method of treating a human at risk to developing, with suspected or with actual macrovascular disease** which comprises or includes the steps of:

(i) **categorising** the human as a candidate patient by reference to

(a) whether suffering from Type II diabetes or impaired glucose tolerance, and

(b) iron status, and/or

(c) heart function

(ii) **subjecting** the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the walls of major blood vessels whilst ensuring (eg; by at least some testing) patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

42. **A method of treatment reliant upon the methodology of any one or more of the accompanying drawings.**

43. **A method of treating a human having Type II diabetes or impaired glucose intolerance at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes subjecting the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron to heart tissue whilst at least on occasions having monitored and/or monitoring the patient to avoid an iron deficit.

44. A method of claim 43 wherein said patient has been categorised to ensure that the regime is not commenced and/or does not continue should the patient be iron deficient and/or suffering from iron deficiency anaemia.

45. A method of claim 43 or 44 wherein the patient is categorised to exclude classical iron overload, ie; the patient to be subjected to the iron chelation regime is one with chelatable iron in heart tissue that may be present above normal levels of such chelatable iron in heart tissues and not one subject to chelation or other therapy to reduce total iron level.

46. **A method of treating a human having Type II diabetes or impaired glucose intolerance at risk of developing, with suspected or with actual macrovascular disease** which comprises or includes subjecting the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the walls of major blood vessels whilst at least on occasions having monitored and/or monitoring the patient to avoid an iron deficit.

47. A method of claim 46 wherein said patient has been categorised to ensure that the regime is not commenced and/or does not continue should the patient be iron deficient and/or suffering from iron deficiency anaemia.

48. A method of claim 46 or 47 wherein the patient is categorised to exclude classical iron overload, ie; the patient to be subjected to the iron chelation regime is one with chelatable iron in the walls of major blood vessels that may be present above normal levels of such chelatable iron in the walls of major blood vessels and not one subject to chelation or other therapy to reduce to iron level.

49. **A method of treating a human at risk of developing, with suspected or with actual cardiomyopathy related heart failure** which comprises or includes reducing the levels of chelatable iron in the heart tissue of such patient without taking the patient into iron deficit.

50. **A method of treating a human at risk of developing, with suspected or with actual macrovascular disease** which comprises or includes reducing the levels of chelatable iron in the walls of major blood vessels of such patient without taking the patient into iron deficit.

51. **A method of treating a human at risk of developing, with suspected or with actual cardiomyopathy** which comprises or includes the steps of

(i) categorising the human as being at risk of developing, with suspected or with actualcardiomyopathy, and

(ii) subjecting the patient to an iron chelation regime with a view to decreasing the presence of chelatable iron in the patient's heart tissue.

52. A method of claim 51 wherein said iron chelation regime is subject to monitoring to ensure the patient does not have or does not develop an iron deficiency and/or iron deficiency anaemia.

53. A method of claim 51 or 52 wherein said categorisation relies on an initial check of heart function and the patient being categorised for the iron chelation regime when that heart function is below normal.

54. A method of any one of claims 51 to 53 wherein the heart function monitoring continues into or beyond the iron chelation regime.

55. A method of any one of claims 51 to 54 wherein said categorisation includes a determination of the patient suffering from Type II diabetes or impaired glucose tolerance.

56. A method of any one of claims 51 to 55 wherein said categorisation involves a reference to iron status of the patient prior to any commencement or substantial duration of the iron chelation regime to ensure the patient does no have or does not develop an iron deficiency and/or iron deficiency anaemia.

57. A method of any one of claims 51 to 56 wherein the regime is with a chelator which in the mammal is substantially without an ability to generate free radicals in significant qualities and which also in the mammal at the dosage regime to be given will not chelate iron down to a depletion state in the mammal.

58. A method of any of claims 51 to 57 wherein the regime is at a dosage regime less than that which for a patient suffering from classical iron overload would have the effect of reducing the iron levels of that patient to normal.

59. A method of any one of claims 51 to 58 claim wherein the regime is at a dosage regime (ie; whether dependent upon dosage unit(s) and/or frequency) which does not or will not reduce a patient of normal iron levels to a deficiency state and/or to anaemia.

60. A method of any of claims 51 to 59 wherein the regime is in concert (serial, simultaneous or otherwise) with a regime to antagonise fructosamine oxidase.

61. A method of claim 60 wherein the regime to antagonise fructosamine oxidase relies upon copper chelation.

62. A method of claim 61 wherein the dosage unit(s) is (are) the dosage unit(s) of the iron chelation regime also.

63. **The use of a compound (a) which itself *in vivo* or (b) which has at least one metabolite *in vivo* which is an iron chelator** for the production of a pharmaceutical composition able to reduce the level or iron in heart tissue and/or in the walls of major blood vessels respectively for the treatment of diabetic, cardiomyopathy and/or diabetic macrovascular disease.

64. A method or use of any one of the preceding claims wherein the chelation is with one or more of the following compounds hereafter referred to:

- trientine.
- a derivative of trientine
- a salt of trientine
- a hydrochloride salt of trientine
- the dihydrochloride salt of trientine
- desferrioxamine
- deferoxamine mesylate
- mimosine
- bathocuproine sulphonate
- bathophenanthroline sulphonate
- 1,2-diethyl-3-hydroxypyridine-4-one
- O-Trensox
- N,N'-Bis(3,4,5-trimethoxybenzyl)ethylenediamine-N,N'-diacetic acid
- N,N'-Bis(3,4,5-trimethoxybenzyl)ethylenediamine-N,N'-diacetic acid diacetoxymethyl ester
- desferri-exochelin 772SM
- deferiprone (1,2-dimethyl-3-hydroxypyridine-4-one)
- diethylenetriaminepentaacetic acid (DETAPAC)

- hydroxyethyl starch-deferoxamine mesylate (HES-DFO)
- desferrithiocin
- 4,5-dihydro-2-(2-hydroxyphenyl)-4-thiazolecarboxylic acid (desmethyldesferrithiocin, DMDFT)
- 4,5-dihydro-2-(2,4-dihydroxyphenyl)-4-thiazolecarboxylic acid [4-(s)-hydroxydesaza-DMDFT
- 2-(2-hydroxyphenyl)-4-oxazolinecarboxylic acid.
- 2-pyridylcarboxaldehyde isonicotinoyl hydrazone
- 2-pyridylcarboxaldehyde benzoyl hydrazone (PCBH)
- 2-pyridylcarboxaldehyde m-bromobenzoyl hydrazone (PCBBH)
- 2-pyridylcarboxaldehyde 2-thiophenec rboxyl hydrazone (PCTH)
- isonicotinoyl salicylaldehyde hydrazone
- 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone (NIH)
- ((+)-3-hydroxy-1-(2-hydroxyethyl)-2-hydroxyphenyl-methyl-1H-pyridin-4-one)
- dexrazoxane (razoxane; 4,4-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione
- CGP 75254A
- N,N'-bis(o-hydroxybenzyl) ethylenediamine-N,N'-diacetic acid (HBED)
- N,N'-ethylenebis(o-hydroxyphenylglycine) (EHPG), and
- 2,3-dihydroxybenzoic acid (2,3-DHB).

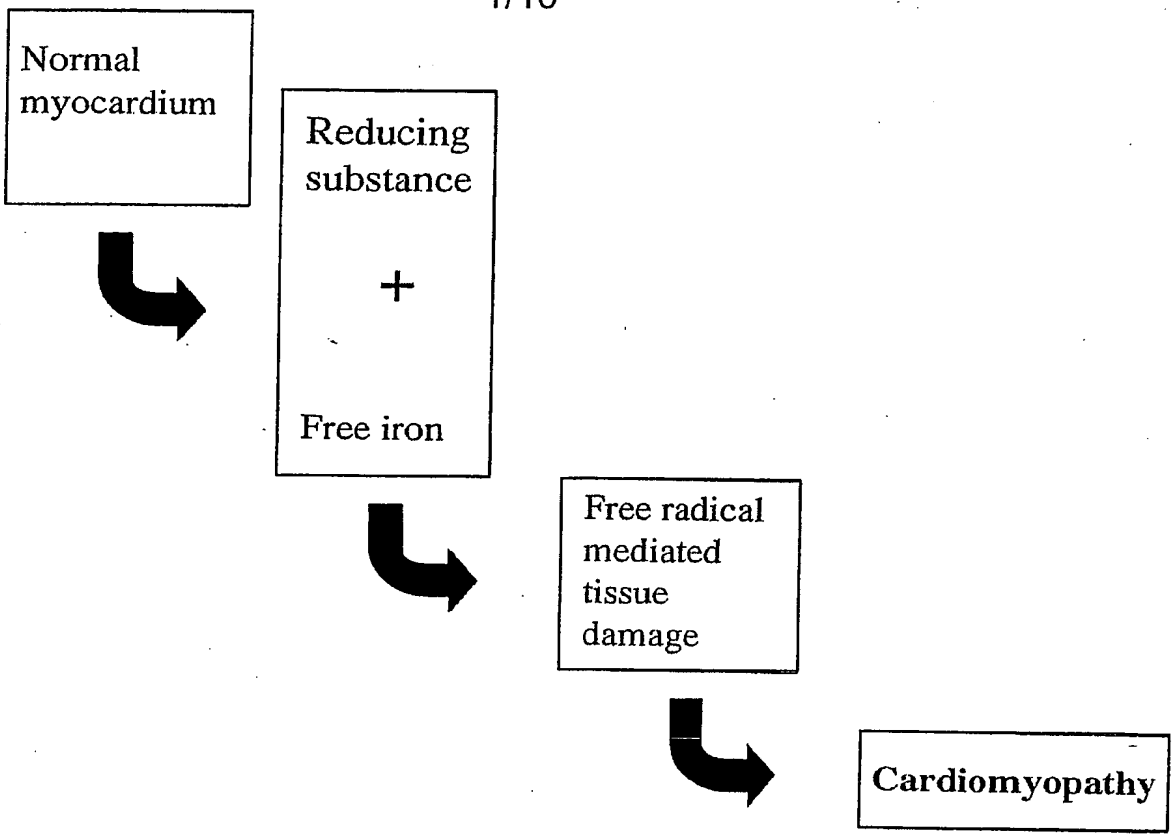


FIGURE 1

Hypothesis

1 ***Fructosamine oxidase***

g-Protein* $\rightarrow$ Protein + O_2^-

2 ***Superoxide dismutase***

$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$

3 **Haber-Weiss Reaction** (Iron salt catalyst)

$H_2O_2 + 2O_2^- \rightarrow O_2 + OH^{\bullet} + OH^-$

* g-Protein = glycated protein substrate

FIGURE 2

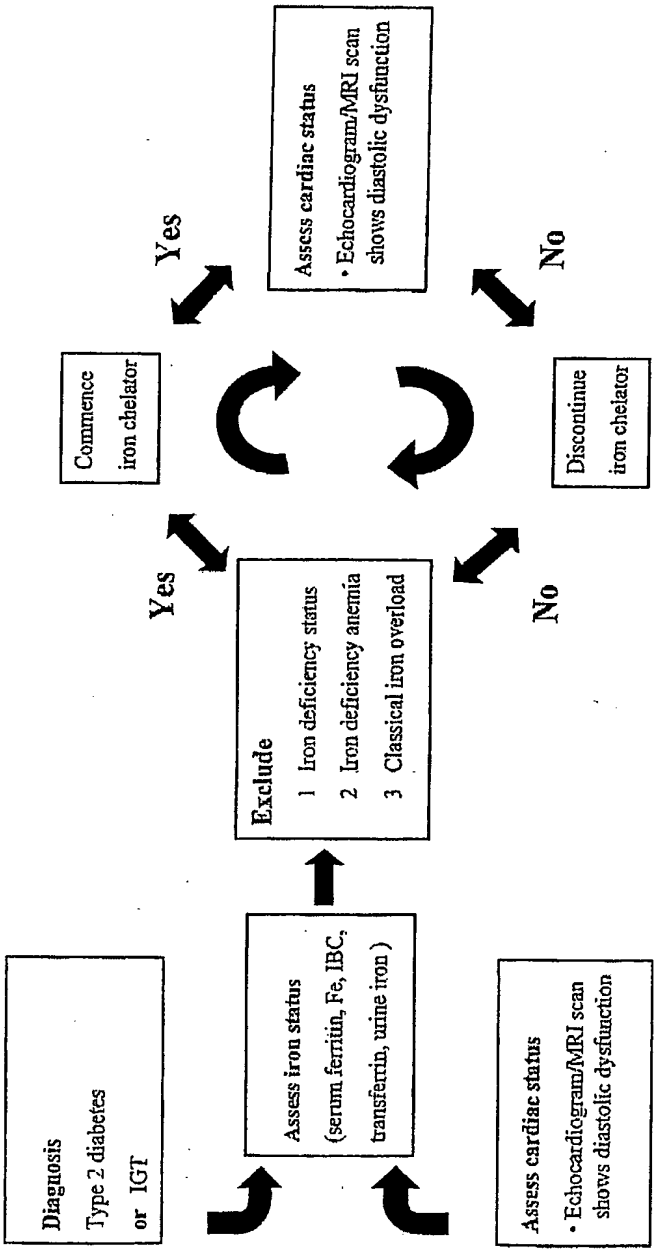


FIGURE 3

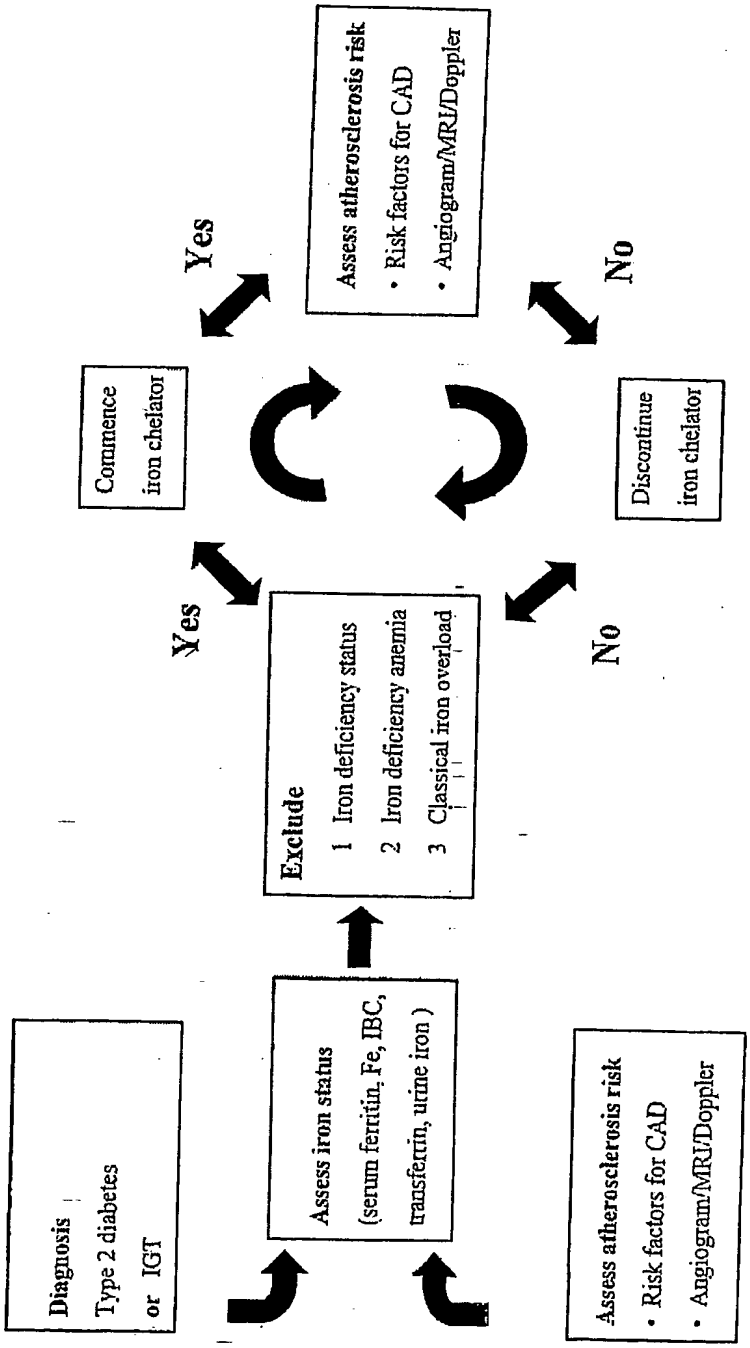
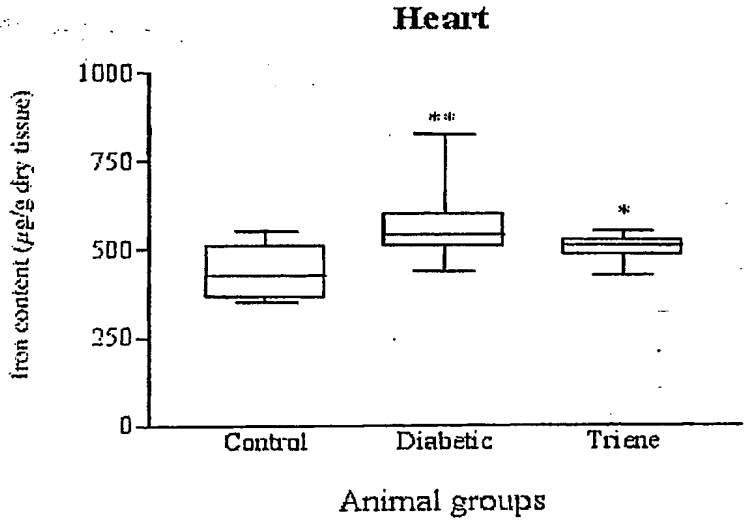


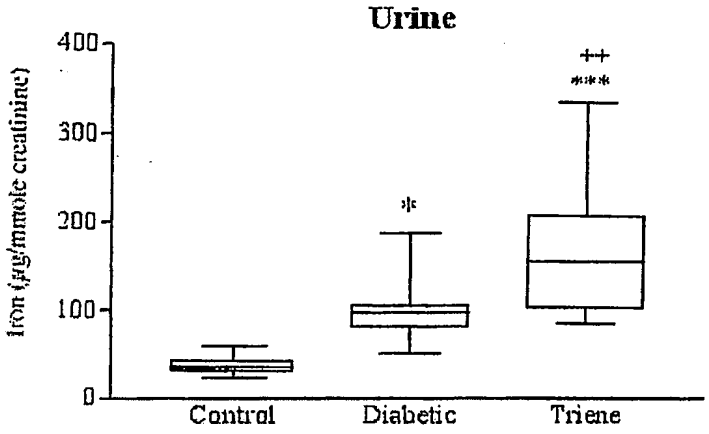
FIGURE 4



ANOVA: P = 0.0041

** P < 0.01; * P < 0.05 versus control.

FIGURE 5



ANOVA: P < 0.0001.

* P < 0.05; ** P < 0.001 versus control group.
++ P < 0.01 versus diabetic group.

FIGURE 6

Grading of Cardiomyopathy

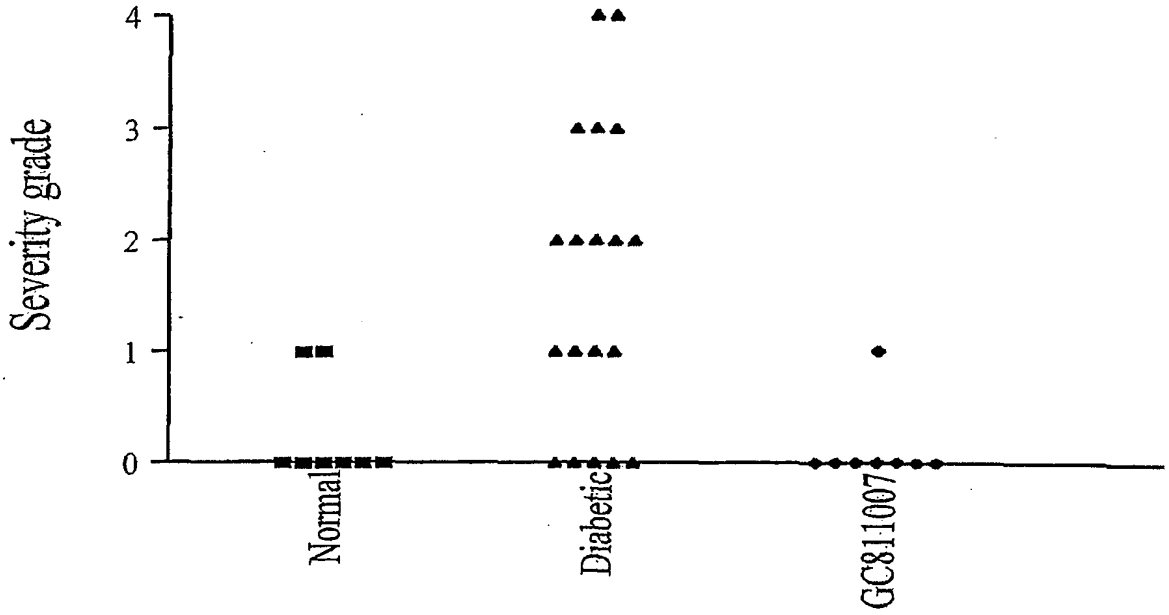


FIGURE 7

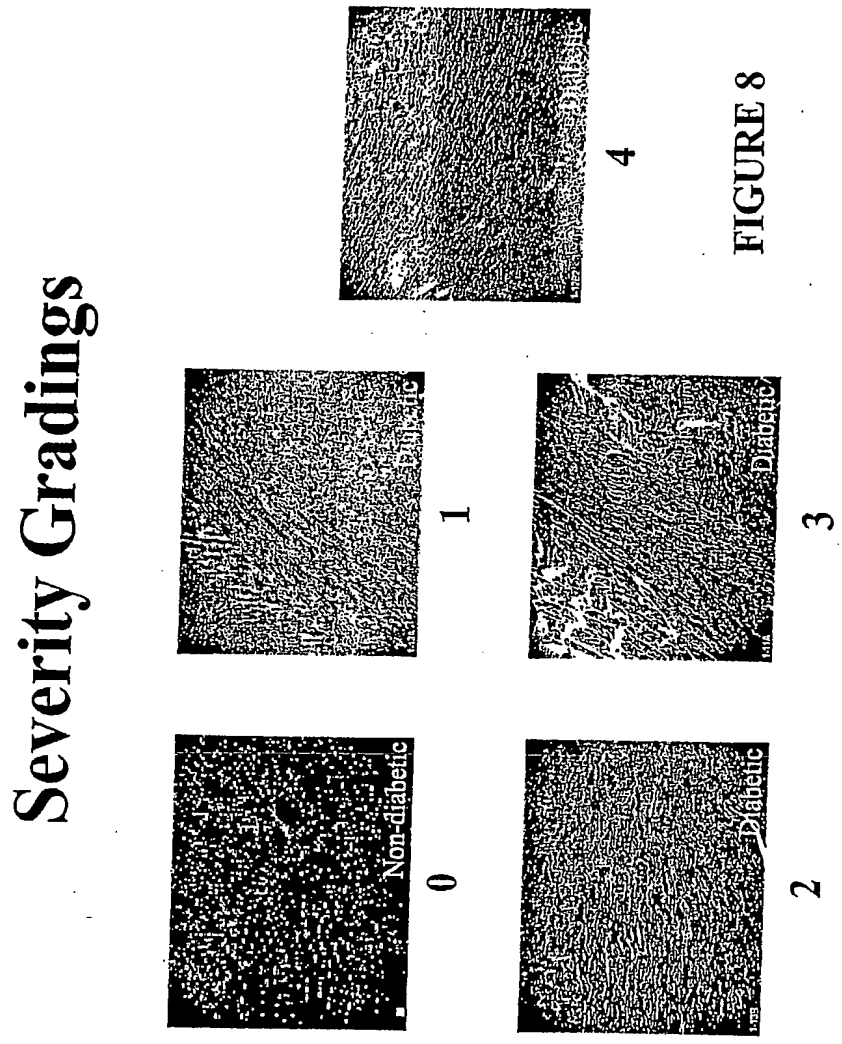
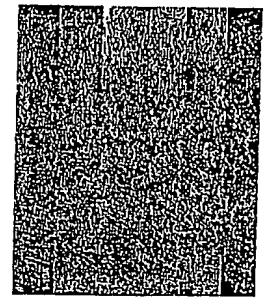
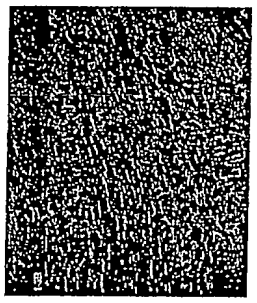


FIGURE 8

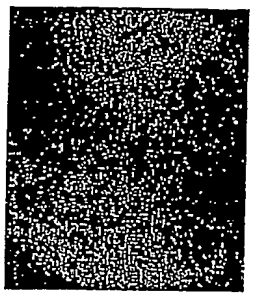
Masson's Trichrome Staining of Heart Tissues



Untreated Diabetic



Non-diabetic



Triene Treated Diabetic

FIGURE 9

Masson's Trichrome Staining Density

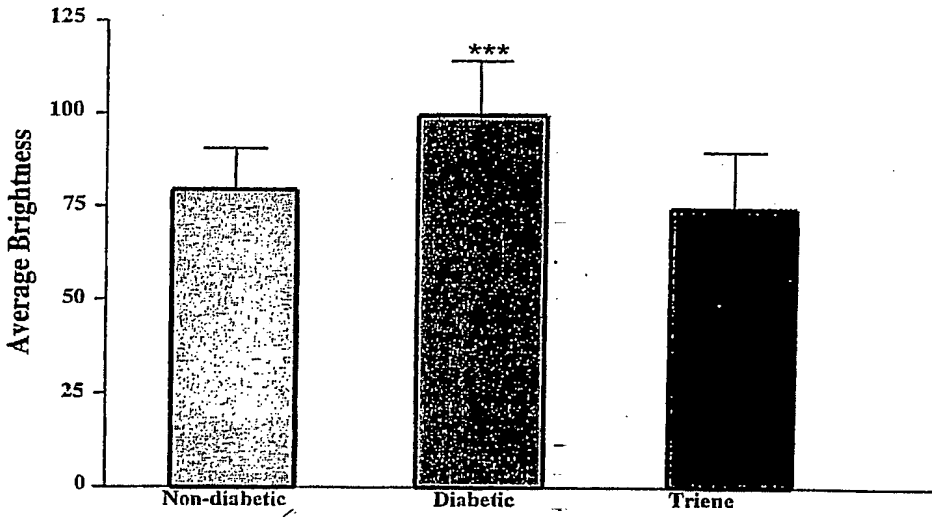


FIGURE 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ03/00043

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 31/198, 31/38, 33/30, 31/45, A61P 3/10, 9/00.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, MEDLINE; keywords:myocard+, cardiomyopathy
+, heart+, hyperten+, ater+, iron, Fe.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6147070 A (Francesco Facchini) 14 November 2000. See whole document.	1-64
X	Howes et al, 'Role of stored iron in atherosclerosis'. Journal of Vascular Nursing, Vol 18 No 4, Dec 2000, pp 109-114. See whole document.	1-64
X	Pucheu et al, 'Effect of Iron Overload in the Isolated Ischemic and Reperfused Rat Heart'. Cardiovascular Drugs and Therapy, 1993, Vol 7 pp 701-11. See whole document.	1-64

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
5 June 2003

Date of mailing of the international search report 17 JUN 2003

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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ03/00043

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Duffy et al, 'Iron Chelation Improves Endothelial Function in Patients With Coronary Artery Disease'. Circulation 2001, Vol 103 pp 2799-2804. See whole document.	1-64

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ03/00043

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
US	6147070	AU	44215/99	WO	9962336

END OF ANNEX